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# *PRINCIPLES OF SALMONID CULTURE*

*WILLIAM PENNELL and*
*BRUCE A. BARTON/EDITORS*

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# PREFACE

As salmonids have been reared for more than a century in many countries, one might expect that principles are well established and provide a solid foundation for salmonid aquaculture. Indeed, some of the methods used today in salmonid rearing are nearly identical to those employed one hundred years ago. Yet scientists are now actively involved in salmonid research in many disciplines, and the rapidly growing literature is becoming so voluminous that it is difficult to keep pace with it. Areas of salmonid research today include nutrition, smolt and stress physiology, genetics and biotechnology, and diseases.

While science sets the ultimate foundations for aquaculture endeavour, technology evolves somewhat independently and usually much more rapidly. Research often follows technological advances building the footings to support the next advances in technology. Experience, 'gut' feeling, trial and error, and 'grapevine' communication may all lead a grower toward certain practices simply "because they work". Later, disciplined research may explain why a particular process was effective and lead to further advances in practice. While not surprising, this pattern leads to difficulties in the preparation of books such as this one. Many important practices may not be well established or even appear in refereed publications. Also it is difficult for scientists to keep up with the rapid and often unpublished changes in the industry's technology.

We have attempted to deal with this problem by setting forth, wherever possible, the principles and major industry protocols for salmonid aquaculture, and to suggest areas where scientific understanding requires more research effort. While this book is not a "how to" manual, it is aimed at a very applied area of work. The purpose of this book is to provide a useful synthesis of the biology and culture of salmonid fishes. Toward that end, we undertook to describe the important practices in salmonid culture as well as the theory behind them. We have assumed that our audience will be diverse and include upper level and graduate students, instructors, researchers, and fisheries biologists and managers. We sincerely hope that practicing aquaculturists also will find the volume useful — after all, they are the experts. The scientific territory of salmonid culture is now so broad that such reviews as presented herein should benefit the people rearing the fish as well as those studying them. Many references to other reviews and texts have been cited throughout this volume for interested readers wishing to explore a particular subject further. Indeed, we encourage readers to do so.

This volume has been much longer in preparation than originally anticipated. Many of the manuscripts were held up for a variety of reasons beyond the control of both authors and editors. Problems of health, departmental budgets and reorganization, changes of career paths, and even fish wars and missing sockeye salmon all created production delays. But finally almost all of the originally planned manuscripts were completed, reviewed, and set into camera-ready copy for the publisher. Those manuscripts written early in the process were either updated or provided with an explanatory note to the reader. Fortunately most of these chapters cover relatively stable areas of the subject and major revisions were not necessary. We were forced to cancel two chapters, but portions of these have been covered in other chapters with key references to source materials.

Chapter authors were solicited on the basis of their expertise in a particular field. As much as possible, we tried to retain the writing styles and presentation formats of those individual contributors. We are very grateful to those many authors for their patience in either putting up with letters and calls from the editors or waiting for their work to appear in print. Also, we would like to thank the many external reviewers who have added their knowledge and perspectives to the chapters.

Very special thanks go to Dr. Donald F. Alderdice who had the original idea for the project and who provided criticism and encouragement throughout its production. Robert Goodman, recently retired from Elsevier, steered the work through its early stages and was very helpful and encouraging throughout the project. Ken Plaxton at Elsevier has been the project's senior editor and Joyce Happee was the head of production; their help has been essential. John Wilson kindly produced the book's cover design.

Brenda Adkins has been a mainstay of this project and we are extremely grateful for her dedication and perseverance. She has been responsible for all of the computer technology and the production of camera-ready copy from manuscripts. She has also kept the editors on track and organized, and kept at a demanding job for far longer than she had probably ever imagined when she began.

We recognize that some facets of salmonid culture are rapidly changing as continuing research leads to new discoveries and technological innovations. Nevertheless, it is our wish that those who acquire this book will find it useful for many years to come.

The Editors
April, 1996

## SPECIAL ACKNOWLEDGEMENTS

Many people were helpful during the planning and production of this book. We would like to especially thank two of these people.

We thank our technical assistant Brenda Adkins for the outstanding job she did preparing all chapters in camera-ready format for publication. Without her computer skills, patience and personal dedication to this project "beyond the call of duty", publication of this volume would not have been realized.

Dr. D.F. (Don) Alderdice, retired from the Pacific Biological Station, first developed the concept for this book, and has been generous with his help and encouragement throughout the project. His advice, based on many years of salmonid research, and his superb editing skills have added greatly to this volume. Any shortcomings are the responsibilites of the editors.

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# INTRODUCTION

The salmonid motif on the book's cover is typical of depictions found in the traditional culture of the aboriginal peoples in the Pacific coastal regions of North America. The frequent occurrence of salmon in the art and totems of these cultures reflects the importance of salmonids in the daily life of these pre-Columbian civilizations. The salmon is still important in coastal native communities but competition for shrinking natural stocks of all species from commercial and recreational interests is very high. Such is the situation globally; hence, the continuing and growing interest in aquaculture of salmonid fishes.

Salmonid culture is now a very substantial activity and very extensive geographically. Hatcheries and related facilities now produce a large proportion of the total wild harvest; in the Pacific Ocean, most of the Columbia River basin's Pacific salmonid runs, about 20% of British Columbia's stocks, and nearly all of the Japanese salmon come directly from hatcheries. Alaska has a very large hatchery program although many more wild runs still exist. Large new hatcheries are being built in Russia and other former Soviet countries. In addition, major runs of sockeye salmon (*Oncorhynchus nerka*) are supported by artificial spawning channels in British Columbia. In the Atlantic Ocean, Atlantic salmon (*Salmo salar*) populations are much reduced from earlier times, but there are many attempts to restore runs through river habitat reclamation and hatchery programs. For example, hatcheries now support most of the Baltic Sea's populations of this species. Clearly a very large proportion of the world's anadromous salmonid populations now emanate from fish culture facilities.

Commercial fisheries for salmonids are thus becoming increasingly reliant on hatchery programs to supplement declining natural resources. Tremendous government efforts and funding have been directed toward substituting depleted stocks with cultured fish to mitigate past effects of overexploitation and habitat loss on natural populations. This reliance on hatcheries is not without risk and controversy. There are valid concerns over genetic effects and many challenges exist in fisheries management, the mixed-stock fishing problem chief among them. Hatchery fish may also greatly complicate fishing treaty negotiations and hatcheries have been accused of making alternate use of river habitats more easily justifiable (for example, hydro-electric power production). On the other hand, hatcheries are now such a major factor in the overall management of wild salmonids that they are permanent features in both the Atlantic and Pacific Oceans and nearby waters.

A similar pattern exists for the various salmonid species exploited for sport in coastal and interior watersheds. The economic benefits of recreational fisheries throughout the world provided by introductions of artificially reared salmonids cannot be denied. Pacific salmon in the Great Lakes, salmon and trout programs in New Zealand, or the very popular, catchable rainbow trout (*Oncorhynchus mykiss*) fisheries in many U.S.

states are but a few of the many examples where stocking salmonids has generated millions of hours of angling enjoyment and substantial revenues to regional economies. The importance of fish culture in recreational fisheries management is reflected by the effort expended by the agencies responsible, the fiscal allotment for the fish culture program often being the single largest line item in a management agency's annual budget. Many government hatchery facilities exist solely to support sport fisheries by producing fish for stocking programs. The same concerns exist over the impacts of these programs on native fish communities as in commercial fisheries, and in recent years stocking programs have been scrutinized and adjusted in an attempt to reduce impacts on wild stocks.

As well as recreational and commercial benefits, the wise use of hatchery stocking programs also has potential in restoration efforts to halt the decline of threatened species, such as the bull trout (*Salvelinus confluentus*) in western North America or the huchen (*Hucho hucho*) in eastern Europe, and specific stocks, such as the Snake River sockeye salmon or the lake charr (*S. namaycush*) in the lower Great Lakes. However, the use of introductions to supplement, restore or enhance wild salmonid populations is not without its critics and for valid reasons. Published literature is replete with "horror stories" documenting the detrimental effects of introductions on natural fish communities through competition, predation, habitat alteration, hybridization, altered reproductive behaviour, and introduction of pathogens. Salmonid culture for management is likely here to stay and, fortunately, most managers now subscribe to the notion that if salmonid stocking is to be undertaken, there are no good excuses for not doing it properly.

Farming of trout and salmon for human consumption has become a very large industry over the last decade. In excess of 200,000 tonnes (metric tons) of trout, mainly rainbow trout, are produced each year in Europe, North America, Japan, and South America. Most trout production focuses on smaller 'portion-sized' fish. In fact, trout are produced in many parts of the world virtually wherever there is water cool enough to support them. Trout farms tend to be small and are usually located near their markets. Trout farming is not a new activity, but has been important for many decades.

The production of large salmonids in sea cages is a relatively recent phenomenon and a major development centre is now established in Norway. The most important sea-cage production takes place in Norway, Chile, the U.K., Canada and Japan, but significant production also comes from New Zealand and Australia. The chief species grown is Atlantic salmon but considerable quantities of chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon are also produced, and recently large rainbow trout have been farmed in increased amounts for export into Japan. This has been a very dynamic industry with dramatic growth in production and concomitant changes in prices. The industries of many countries have been through drastic reorganizations. As a result, the emphasis is now on lowering the cost of production as prices fall while simultaneously expanding into new markets, sometimes with new products. Virtually all Atlantic salmon on the market are produced by sea-cage operations, and farmed salmon and trout now account for just less than one-half of the total commercial salmon production in the entire world.

In the early days of salmon sea-cage farming, it was difficult to imagine that hand-fed fish, exposed to risk of disease and accident, could ever compete with wild-caught fish. However, farmed fish have a much higher value than wild fish because they can be produced throughout the year and delivered to markets consistently in a fresh, high quality form. By contrast, wild fish tend to become available all at once, usually in late summer and fall as anadromous runs enter coastal waters, and often in a highly variable species mix. This harvest depresses prices, and the quality of these fish, in terms of careful processing and shipping, has rarely matched that of the farmed product. Much of the wild harvest traditionally was canned. Consequently, farmed salmon have been able to compete very successfully with wild harvests. The intricacies of marketing this product are well discussed in Chapter 15.

During the last decade, total global salmon production has increased. The Pacific Ocean provides most of the world's wild harvest and overall production has been on the increase. This is in part due to successful ranching programs in Pacific rim countries, but is also due to oceanographic conditions that have apparently contributed to better growth and survival of open sea stocks. The effect of these increases, combined with the rapid expansion of farmed fish production, has been to depress world prices significantly. Lower prices have placed enormous pressure on the salmon farming industry to lower production costs. The current salmon farm situation is often compared to the early days of industrial poultry production and much of the research now being done on salmon culture is aimed at reducing production costs.

To provide as broad a foundation as possible for salmonid culture, the book begins with an historical review in Chapter 1 and a discussion of general salmonid biology in Chapter 2. This is followed by an extensive review in Chapter 3 of salmonid life histories that describes the patterns underlying the vast complexity and plasticity shown by these fishes. The intention of Chapters 2 and 3 is to present the reader with a context and background for the remaining chapters and to show something of the limitations and opportunities set by the basic biological material with which the culturist must work. As an example of the importance of basic fish biology to culture, it is now becoming clear that the behaviour of fish in intensive aquaculture situations, which has been ignored until recently, offers great potential for improvement in fish performance under production conditions (*see* Chapters 3 and 6). Also, thorough knowledge of early developmental biology is essential for establishing fertilization techniques and incubation protocols that will produce the highest quality products (*see* Chapter 5).

The nutritional requirements of salmonids are discussed in Chapter 10. Diet formulation is central to cost of production in terms of growth and conversion rates, feed costs, and fish health. Salmonid nutrition research has a significant history and the basic requirements are now well established for most of the species reared. However, more work is needed in specific areas, for example, on lipid to protein ratios in feed formulations for on-growing. Recently, very high lipid levels have been successful in increasing growth rates in Atlantic salmon. Although these lipid levels would not appear to be greatly higher than those in the natural diet, they are much higher than traditional formulations and appear to pose some difficulties with Pacific salmon. Notably, much work in nutrition research is now done by large feed companies and is usually of a proprietary nature, while public-domain research catches up over time.

Another area of salmonid nutrition of great interest is the preparation of proteins from plant sources as substitutes for marine or animal-based protein. While it has been axiomatic in fish nutrition that fish-based protein provides the best growth rates, recent work suggests that land-based proteins, such as from seeds and legumes, when separated from plant carbohydrate and phytotoxins, can produce reasonable growth in rainbow trout. If this approach becomes commercially viable it will have the effect of moving salmonids well down the trophic pyramid. In the long term, this shift could greatly reduce costs of production as well as stabilize feed sources.

Feeding strategy also appears to have a very significant effect on fish performance in both the freshwater hatchery and in later on-growing. This is discussed in Chapters 6 and 9. The delivery of feed in a manner to reduce agonistic behaviour and provide a nearly equal ration to all fish is almost as important as providing a good formulation. The study of fish behaviour in intensive rearing is relatively recent and will likely be very helpful in attaining improved feeding strategies. It may also be important to feed fish at certain intervals corresponding to endogenous rhythms and to be aware of gut evacuation rates. Chapter 7 discusses a growth model that can be used to assess the influence of various fish culture variables on fish performance. In that chapter, interesting work is reviewed on compensatory growth phenomena. By alternating periods of fasting and feeding, it may be possible to attain increased feed conversion efficiencies while maintaining growth and reducing labour and feed costs.

For fish to grow well, they must be healthy. A host of bacteria, viruses and parasites cause problems in intensive culture facilities. Discussion of these pathogens and their modes of expression is presented in Chapter 13. It is usually not possible to maintain hatcheries absolutely free of disease, but there are techniques available to greatly reduce pathogen levels in freshwater facilities. By producing fingerlings or smolts with very low pathogen incidence, the chances for on-growing without an epizootic are increased. Presumably survival in the wild environment will be increased through the same measures.

In the natural environment pathogen challenge is inevitable, but stress reduction combined with the development of vaccines will usually result in successful disease management. As veterinarians point out, disease is not synonymous with infection. As better understanding of stress physiology develops, reduced stress levels in fish should lead to greater disease resistance. Moreover, a better understanding of fish behaviour may lead to lower stress levels in cultured fish populations. Both stress physiology and vaccine development are important and are active areas of research.

Smolt quality is a key issue in both the cost of production of farmed salmon and the success of ocean ranching. Typically in the sea-cage operation, smolts are delivered to a saltwater site with little opportunity to acclimate to the hypersaline environment. Hypo-osmoregulation must begin immediately or the result will be stunted fish or delayed mortalities. Increasingly, private hatcheries are manipulating the smolt cycle to produce smolts at times of the year other than spring and at a variety of ages and sizes. This has the effect of increasing hatchery cash flows and allowing farms to produce harvestable fish throughout the year. Research in smolt physiology, reviewed in Chapter 8, has made this possible. Smolt research is very active throughout the world and is rapidly expanding our knowledge of this complex process.

In a sea-ranching context, ocean survival is the key issue. While smolt quality is assumed to be a central variable, the influences on smolt success are complex and include changes in the ocean environment. Studies on rearing density and water quality discussed in Chapter 6 are revealing, and much further work needs to be done before smolt releases attain their full potential. In addition to the smolting process, release hatcheries must take into account fish behaviour in captivity. In the hatchery, fish see only conspecifics in the water and may learn to associate above-water movement with food. After release into a natural environment, these fish will have to avoid or escape predators. Stamina involving both energy stores and muscle development may also be important to ocean survival of fish in sea ranching.

Another salmonid culture activity linked to both smolt production and stress management is the transportation of fish. Chapter 12 reviews the techniques used to handle and move fish. It is clear that poor transportation events may degrade the quality of the best smolts, and that the combined stressors of transportation and transfer to a new osmotic regime may be very debilitating unless handling is done carefully.

Selection programs are critical to both farming and release operations. In salmonid farming, cost of production, as affected by disease resistance, growth rate, maturation schedule and feed conversion rate, are goals of deliberate selection programs. Other aspects of carcass value may also be included such as pigmentation and body shape. For release programs, the goal is to have as little artificial selection as possible so that the potential for altering gene frequencies of natural populations is minimal. Chapter 11 reviews both topics in detail and Chapter 4 discusses aspects of selection and fertilization in broodstock programs. The success of carefully designed selection programs is impressive. When developments in nutrition and husbandry are linked to breeding programs, time to harvest is shortened progressively, usually to an extent unimagined at the start.

While domestic fish selection programs are complex and expensive, it is ever more challenging to enhance a wild population (or proportion of a wild population) without seriously altering the genetic nature of the stock. As made clear in Chapter 3, wild spawning behaviour and mate selection are complicated processes and likely impossible to duplicate in the hatchery. There are, however, measures that can minimize genetic effects, especially for short-term hatchery projects. These are discussed in some detail in Chapter 11. This subject is an important area of research considering the large proportion of wild stocks now supported or augmented by hatchery releases.

Release hatcheries have come under increasing criticism from fisheries managers and geneticists in recent years. Hatcheries with poor ocean survival records are being closed down in many parts of the world and private salmon ocean ranching has never been very successful or politically popular (*see* Chapter 14). Hatchery programs have a political side as well, as shown by recent Canadian-U.S. salmon treaty negotiations. Although, as noted above, hatchery ranching programs are likely permanent features of this resource, they will now be scrutinized with more rigor for benefit-cost ratios and possible negative impacts.

The most controversial side of salmonid culture is the fast growing and relatively recent sea-cage culture industry, which has added so much value to the world resource but has also contributed to so much change in its nature. In some countries, salmon farming has been seen as a direct threat to wild stocks and to the fishing industry through the perception that local salmon farms will lower prices for wild fish. Other groups with concerns over aquatic environments have raised water quality issues. Chapter 16 reviews the environmental effects of salmonid hatcheries and grow-out cages from a scientific perspective. Areas of concern include hyper-nutrification, increased eutrophication (especially in freshwater systems), alteration of bottom sediments near cages, and problems caused by the fish themselves. The latter include risks of colonization by non-native species, genetic impacts on wild conspecifics, and introduction of pathogens into wild populations. Clearly some of these issues remain theoretical and the subject of risk analysis, but the intensity of some debates suggests an emotional involvement that departs from scientific evidence. Yet such debates are of vital importance to the industry, especially salmon farming, as opposition to salmon farming has often led to moratoria on new site development or even legislation against all farming activity. The real and important issues must be scientifically separated from false or trivial issues and efforts are needed to solve or avoid potential environmental problems.

Chapter 17 discusses the role of biotechnology in salmonid culture. This is perhaps the fastest moving area of research and one that has a very great potential for increasing productivity of salmonid farms. It is also an area that generates controversy. For example, use of growth hormone has been shown to increase the overall performance of salmonids in terms of growth rate and feed conversion. It is a safe practice posing no apparent risk to the consumer. However, it remains doubtful that the public will accept this practice and the use of direct hormone treatment opens the salmonid culture industry to criticism by those who oppose it. Transgenic fish with enhanced production of endogenously produced growth hormone may therefore be an acceptable approach for producing faster growing fish. This technique may also be useful for accelerating the progress of normally lengthy and expensive breeding programs.

The development of DNA probes for sex determination offers a technique that may facilitate a rapid development of all-female stocks as an approach to the grilse or 'jack' problem in some species and strains, and for the creation of all-female triploid fish that are useful for controlling grilsing and in meeting certain regulations regarding non-native fish species. Many other applications of biotechnology are discussed in Chapter 17, which concludes the volume.

Making predictions about future salmonid stocks and salmonid culture activities is difficult as these topics are very complex. It does appear that the price of salmon will continue to fall and that salmon farming will increase in magnitude. This trend prediction assumes that market development moves forward rapidly enough to control price reductions (*see* Chapter 15) and that farmers can continue to reduce the cost of production through research and technology. It also assumes that there will be no serious natural disasters in salmon farming countries. Strong and rational regulation of aquaculture industries is vital as the devastating example of shrimp farming problems in Taiwan has dramatically shown recently. New diseases will certainly appear as will new approaches to their management. Stocks of fish will be improved through breeding programs and through biotechnology. Diet formulations will also improve and if the development of land-based protein for fish feeds continues, commercial feeds should decline in cost. All of these developments will probably not move forward in perfect synchrony and periods of adjustment, sometimes dramatic, will likely occur. The continued existence and growth of salmonid culture will require careful attention to environmental impacts in order for the industry to demonstrate to its critics that salmonid farming is ecologically sustainable.

Wild stocks will continue to be augmented by hatchery programs and some endangered genetic strains could be saved by careful application of hatchery technology. Improved management of both hatchery and wild stocks could reduce current reservations about the value of ocean ranching programs, but there is a strong political component to wild stock management, especially when stocks migrate into or through international and multiple territorial waters. Moreover, the possibility of global climate change has enormous implications for the future of salmonid stocks and hatchery programs. What those are, of course, are not currently predictable.

William Pennell and Bruce Barton

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## Chapter 1

# HISTORY AND APPLICATIONS OF SALMONID CULTURE

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## HISTORICAL PERSPECTIVE

Salmon, trout and charr, belonging to the genera *Oncorhynchus*, *Salmo* and *Salvelinus*, are native fish of the cool waters of the northern hemisphere. All spawn in fresh water and many migrate to sea where growth is rapid because of the better availability of food.

The chief characteristics of the commercially important species of salmonids are reviewed by Laird and Needham (1988, 199;, and *see* Chapters 2 and 3 and Table 1). As well as their close biological relationship and ecological similarities the salmonids have a significant common feature: all are highly regarded as food for man and have been so since prehistoric times. Prehistoric paintings in France and North America clearly show salmonid fishes; the seasonal timing of the major salmon runs provided welcome food before the onset of winter. Certain milestones in the understanding of the biology of salmonid species can be identified. These have made possible the management and culture operations which we know today.

The earliest written mention of salmon is generally held to be by Pliny the Elder in *Historia Naturalis*, written in the 1st Century AD, where the name *Salmo* was used for the first time. The general pattern of the life history of the Atlantic salmon was known to Hector Boece, the first Principal of the University of Aberdeen who described the spawning migration, spawning behaviour and emergence of fry accurately (Boece 1527). He also gave a detailed account of poaching, describing the placing of cauldrons of boiling water at the foot of waterfalls to catch salmon that failed in their attempts to ascend.

Boece (1527) included one major mistake in his outline of the life cycle; he estimated the period of seawater residence of salmon to be 20 days from leaving fresh water to returning as full-sized salmon. This misunderstanding continued until the nineteenth century. Similarly, there was a general belief that parr were a separate species of fish, perhaps a kind of trout, and that young Atlantic salmon migrated to sea soon after emerging from the gravel in which eggs had been deposited.

## EARLY ATTEMPTS AT SALMONID CULTURE

Several pioneers carried out experiments which improved our understanding of salmonid life cycles and made culture of the species possible. One of the first of these was Dom Pinchon, a 14th-century monk from the Abbey of Réome, who is said to have fertilized eggs and hatched them by burying them in wooden boxes in gravel (Francis 1865). Similar trials were carried out in Germany in 1763 by Lieutenant Jacobi, a "German Gentleman" (Francis 1865). Salmon and trout eggs were placed in a long, oak box with fine gratings at the top and ends. The box was partially filled with gravel and eggs which had been stripped from female fish and fertilized with milt from males placed in the box. The box was then placed in a clear running stream of suitable depth for incubation to proceed.

The true founder of salmonid culture is commonly agreed to be Shaw, whose experiments and observations made in ponds at Drumlanrig, Scotland, provided definitive proof that parr were the young of Atlantic salmon and could be grown to the smolt stage in fresh water (Shaw 1836, 1840). Over the years, Shaw's experiments included the growing of parr collected from the river, the collection of naturally spawned eggs and artificial fertilisation. Shaw's work demonstrated the importance of conserving parr and their environment as a means of safeguarding the runs of adult salmon.

At about the same time as Shaw was busy in Scotland, two Frenchmen, Gehin and Remy, in the Vosges region, were developing techniques of artificial fertilisation and incubation of salmonid eggs as a means of augmenting depleted stocks. They developed the technique of transporting eyed ova in special containers; from their hatchery on the Rhine, eggs of various salmonid species (trout, charr, huchen) were sent throughout Europe (Francis 1865).

Some experimental rearing produced "results" that appear somewhat curious in the light of our present knowledge of salmon life cycles. Buist, working at Stormontfield on the River Tay, Scotland, reared salmon smolts and marked them with fin clips, opercular punches and silver ring tags and recorded that these fish returned as "grilse" (weighing over 1.5 kg), 3-4 months later (Brown 1862). Grilse are now known to spend at least 14 months in seawater before return to fresh water.

**TABLE 1**

Characteristics of the major commercially important salmonids (from Laird and Needham 1990).

| **Species** | **Common names** | **Native range** | **Time spent in sea water** | **Weight at maturity** | **Migration pattern at sea** | **Potential for farming** |
|---|---|---|---|---|---|---|
| *Salmo salar* | Atlantic salmon<br>[a] Ouananiche<br>[a] Sebago<br>[a] Blanklax<br>[a] Blege<br>[a] Smablank | North Atlantic from New England to Ungava Bay on the west, Iceland, Greenland and from northern Portugal to the Kara Sea on the east | 1 to 5 yrs | 1 to 30 kg | Long distance migrations to mixed stock feeding grounds, e.g., west Greenland | Excellent. High commercial value, relatively easy to handle. Can be farmed outside native range, e.g., Tasmania, British Columbia |
| *Salmo trutta* | Sea trout<br>[a] brown trout<br>many local names for the "jack" form which spends only a few months at sea, eg. finnock, whitling, sewin | Sea-going forms are found in countries bordering the northeast Atlantic where maximum sea temperatures are <21°C. | Few months to 3 yrs then 1 to 2 yrs between spawnings | 300 g to 10 kg + | Coastal, feeding in shallow inshore waters and estuaries | Limited, mainly for stocking for leisure fishing |

TABLE 1 CONT'D.

| Species | Common names | Native range | Time spent in sea water | Weight at maturity | Migration pattern at sea | Potential for farming |
|---|---|---|---|---|---|---|
| *Oncorhynchus mykiss* (formerly *Salmo gairdneri*) | steelhead<br>[a] rainbow trout<br>Kamchatka trout | western North America from Mexico to the Bering Sea, Siberia | <1 yr to 4 yrs | 200 g to 19 kg | Coastal or long distance migrations | Can be farmed in fresh water or in seawater installations similar to those used for Atlantic salmon but, in most sea sites, is less financially attractive |
| *Oncorhynchus gorbuscha* | pink salmon<br>humpback,<br>humpy<br>karafuto-maru<br>gorbuscha | East and west Pacific | Always 2 yrs | 1 to 5 kg | Long distance elliptical migration route of over 300 km | Most abundant of all Pacific salmon in the wild. Fry migrate to sea soon after hatching, reducing rearing costs in ranching operations |

TABLE 1 CONT'D.

| **Species** | **Common names** | **Native range** | **Time spent in sea water** | **Weight at maturity** | **Migration pattern at sea** | **Potential for farming** |
|---|---|---|---|---|---|---|
| *Oncorhynchus keta* | chum salmon<br>dog, sake<br>keta | East and west Pacific. Widest distribution of all the Pacific salmon species | 3 to 4 yrs, 5 for Yukon chum | 3.4 to 4 kg, 20 kg for Yukon chum | Long distance, several 3000 km circuits of the Pacific | Poor flesh colour and quality. Fry usually migrate to sea; this is exploited in the Japanese ranching program. Poor survival in cages |
| *Oncorhynchus nerka* | sockeye salmon<br>red, blueback<br>beni-masu<br>nerka<br>[a] kokanee<br>[a] kikaninny<br>[a] little redfish | East and west Pacific in rivers with lakes in the system. Greatest numbers from Bristol Bay to the Columbia River in the east, Kamchatka in the west | 3 yrs or more, males may mature as jacks | 1 to 5 kg | Long distance except for jacks which remain in coastal waters | Good flesh colouration comes from the crustacean diet. Disease-prone in captivity. Spawning channels used to enhance natural stocks |

TABLE 1 CONT'D.

| Species | Common names | Native range | Time spent in sea water | Weight at maturity | Migration pattern at sea | Potential for farming |
|---|---|---|---|---|---|---|
| *Oncorhynchus kisutch* | coho salmon<br>silver<br>gin-maru<br>kizhuch | East and west Pacific from coastal California north to Norton Sound, Alaska. Hokkaido (rare) to the Anadyr River | 2 yrs except for jacks | 4 to 5 kg | Usually remains in coastal waters | Disease-free eggs are available, making this the preferred Pacific salmon for farming in Japan, USA, Chile and Canada. Remains in the vicinity of net cages if released, providing a local sport fishery |
| *Oncorhynchus tshawytscha* | chinook salmon<br>king, spring<br>quinnat<br>masunosuka<br>chavycha | East and west Pacific, Ventura River California to Point Hope, Alaska. Hokkaido to the Anadyr River | 1 to 5 yrs normally, 6 to 7 yrs for Yukon females | Avg. 10 kg maximum 55 kg | Coastal | Widely reared in fresh water for enhancement of depleted stocks in North America. Reared in cages in Canada, New Zealand and Chile |

**TABLE 1 CONT'D.**

| Species | Common names | Native range | Time spent in sea water | Weight at maturity | Migration pattern at sea | Potential for farming |
|---|---|---|---|---|---|---|
| *Oncorhynchus masou* | masu, cherry yamame, sima | West Pacific, over the southern part of the range of the other Pacific salmon species | 1 to 2 yrs, jacks <1 year | Avg. 4 kg except for jacks | Coastal | Propagated in Japan to sustain natural stocks |

[a] Landlocked form

In the later part of the nineteenth century salmonid hatcheries were established in both Europe and North America. There were two main reasons for this development; the recognition that natural stocks of salmonids were in decline (it was assumed that the progeny of artificially spawned fish would be better than that of wild fish [Thorpe 1980]) and the desire to introduce salmon and trout to new parts of the globe. Brown trout introductions were often successful, at least in the short term. The first introduction of brown trout outside its range took place in 1864 to the Plenty River, Tasmania, with eggs sent from England by Frank Buckland and Francis Francis, notable pioneers in this field (Buckland 1863; Francis 1865). Around 300 eggs survived the long sea journey. These were supplemented by later shipments and a breeding population became established. Sir James Maitland of Howietoun near Stirling in Scotland established a hatchery for rearing the Loch Leven strain of brown trout. This hatchery (which is still operating) was copied throughout the world (Maitland 1887). The success of this movement can be judged by the fact that brown trout (natives of Europe and southwest Asia) have been established in every continent with the exception of Antarctica. Brown trout have even become naturalized in tropical countries such as Kenya and Uganda where they are found in the cooler mountain regions, up to 1200 m above sea level. The sea-going form of the brown trout, the sea trout has also become naturalized but in fewer places: British Columbia, New Brunswick, Nova Scotia, Newfoundland and the Falkland Islands. A general rule with all salmonids seems to be that the chances of successful introduction are higher with non-anadromous than with anadromous forms.

Rainbow trout from the west coast of North America were first introduced outside their native range in 1874 when McCloud River stock were transferred to the Caledonia River, New York. Since then they have been transported around the world in a similar manner to brown trout and many self-sustaining populations have developed, including sea-going populations in Prince Edward Island, Canada (MacCrimmon 1971). The rainbow trout farming industry developed in Europe in the 1890s, with Denmark taking the lead.

By the end of the 19th century there were 18 salmon hatcheries in Scotland; the salmon parr were fed largely on mincemeat and offal. The first experiments on keeping and feeding salmon in seawater (on a diet of chopped liver) seem to have taken place in ponds at the mouth of the Spey at the beginning of the 20th century. This was an important breakthrough, showing that salmon could be kept successfully in captivity in seawater.

Salmonid farming began in North America at around the same time as the European movement was gaining momentum. The first salmon hatchery was built at Newcastle, Ontario, on a tributary stream of Lake Ontario in 1866 by Samuel Wilmot. This was a private venture, perhaps the first attempt at commercial salmon ranching (Kirk 1987). However, the government decided that this project should not go ahead as a private venture but should be publicly funded. More than 1 million Atlantic salmon smolts were produced over the next few years; unfortunately however, the Lake Ontario population was wiped out by pollution. Nevertheless, the initial success of this hatchery had firmly established the principles of stock enhancement through hatcheries; many government hatcheries were then constructed in Canada and the U.S. based on this model. The Craig Brook salmon hatchery opened at Orland, Maine on the Penobscot River in 1871 (Atlantic salmon) and the Baird hatchery on the McCloud River in northern California was built by the U.S. Fish Commission in 1872. This hatchery originally aimed to produce chinook salmon for stocking Atlantic streams; at this time there was still no clear idea of the difference between Pacific and Atlantic salmon.

There was great optimism surrounding the hatchery program at this time. It was generally held that the more eggs and fry released, the more adult salmon would return. Hatcheries were therefore constructed in British Columbia and all the Pacific seaboard states of the U.S., including Alaska where there were massive natural runs of Pacific salmon and steelhead trout. However, by 1900 there was considerable alarm at the high exploitation rate of the Pacific salmon fishery which had increased from an annual pack of 100,000 48-lb cases in the late 1870s to over 2,000,000 cases in 1900 in British Columbia alone. The U.S. government passed legislation requiring canneries to operate salmon hatcheries, as some way of compensating for the fish removed. Tax incentives were later offered for canneries which released ten fry for every adult caught. However, the hatcheries concentrated on rearing and releasing fry of sockeye and coho salmon, both of which remain in fresh water for one or more years before migrating to sea. Survival was therefore probably very low and the exercise of little benefit: there were no follow-up operations to check on the benefits or otherwise of the stocking policies.

The Craig Brook hatchery on the Penobscot River provided the model for the first salmon hatchery to have been built in Japan, which was constructed in 1889 on the headwaters of the Ishikari River in Hokkaido (Kobayashi 1980). It was designed to supply eggs to other hatcheries and became the centre of the Hokkaido hatchery system. By 1910 there were 59 salmon hatcheries in Japan, mainly small and financed either by the government or privately. By 1934 the Hokkaido government had taken over most of these hatcheries (*see* Chapter 14).

## THE ROLE OF CULTURE IN INTRODUCTIONS

The move to introduce salmonids outside their native range has already been identified as one of the driving forces in the development of culture systems. There have been attempts at introduction of the different species in many parts of the world; reviews of the success have been given by MacCrimmon (1971). Often, attempts are heralded as being successful when adult salmon or trout return to spawn but the species may not survive in its new home for more than a few generations unless enhancement is continued. Some examples of successful and unsuccessful introductions are given below. The introduction of salmonids to New Zealand has been one of the most successful of all such operations (Waugh 1980). First introductions took place in 1868 with Atlantic salmon eggs taken from the Severn and Tay Rivers in the U.K. and received in Otago in the South Island by the National Acclimation Society. Five hundred eggs survived the voyage and were planted into the Clutha River. Shipments of salmon and brown trout eggs continued until 1911; the government took over control of the program in 1908, restricting releases of fry to the Upukerora River, a tributary of Lake Te Amuin the South Island. Atlantic salmon have become established in a landlocked form; artificial stocking, however, has always been necessary in order to maintain stocks.

The introduction of chinook salmon (or quinnat, the particular North American name which has been adopted in New Zealand) has been a greater success. The first introductions were made in 1875, by the Hawkes Bay Society, with eggs from California. These failed in the North Island but became established in the Waimakariri River of the South Island and spread to other east coast glacial streams. In 1901 a government policy for the intensive stocking of quinnat was implemented; 50,000 eggs were delivered from California that year and distributed between the Hakataramea River and Lake Ohay hatcheries. Instead of stocking streams with fry with a poor chance of survival, yearlings (23,000) were released in 1902 and two-year olds (23,000) in 1903. Sea-run fish returned in 1906 and 1907; these were stripped and the eggs reared in hatcheries for release as juveniles. By 1910 the program had become so successful that surplus ova were available for export to Tasmania. Quinnat salmon now form the basis of a leisure fishery in New Zealand. They reach weights of 20 kg and have changed their seasonal maturation pattern to fit the southern hemisphere. Quinnat salmon may have colonized more successfully than Atlantics because they remain in coastal waters during their marine phase; Atlantic salmon naturally undergo migrations of several thousand kilometres to mid-Atlantic feeding grounds.

A consignment of sockeye salmon eggs was received in New Zealand in 1901-02 and 150,000 of these were hatched at the Hakataramea hatchery. No further introductions have been recorded but sockeye have become established in fresh water.

There have been many other attempts to introduce Pacific salmon outside their native range. The Russians have made considerable efforts to establish runs of Pacific salmon in the White and Barents Seas. The first attempts, between 1933 and 1938, were with chum salmon from Sakhalin Island which were incubated in a rearing unit at Murmansk. Chum salmon have a short freshwater juvenile phase; fry were released in coastal streams in the anticipation that they would move rapidly to sea. A small number of adults returned to fresh water but the experiment was discontinued as a failure. A later, massive experiment was carried out in the area from 1956. Over 20 years, 200 million pink salmon fry were released from hatcheries and by the 1970s up to 240,000 adults were returning in some years. (Bakshtansky 1980). Stray fish from these releases turned up in salmon nets on the coasts of Norway and Scotland. Natural spawning became established and in some years, natural survival seems to have been high although homing to release sites was poor. The main benefit of such a program would therefore seem to be for fishermen in coastal waters rather than within the rivers.

In North America, natural populations of Pacific salmon are all found west of the Continental Divide. One of the most ambitious programs for the establishment of new populations of salmon was that of the Great Lakes introduction of chinook and coho salmon which took place in the mid-1960s. The natural fish population had been irreversibly damaged by a combination of pollution and the opening of the Welland Canal, allowing ships access from the lakes to the St. Lawrence and the sea. One of the major casualties had been the lake trout, (*Salvelinus namaycush*), which had been destroyed by the sea lamprey (*Petromyzon marinus*). Pink salmon were first accidentally introduced to a tributary of Lake Superior in 1956 when between 100 and 350 fry escaped as a floatplane was being loaded to take them to Hudson Bay (Heard 1991). This was supplemented with the release of 21,000 fingerlings; by 1979 this had given rise to 11 generations, all completing their life cycle in fresh water, and had spread to tributaries of Lake Huron.

In 1966, 660,000 hatchery-reared coho salmon smolts were released into the Huron River leading into Lake Superior (Heard 1991); this produced greater returns to Lake Michigan than to Lake Superior. Even though all the smolts had been released into one stream, returns were spread over 39 rivers and streams, perhaps suggesting poor homing behaviour as also found in the Russian trials.

## THE ROLE OF CULTURE IN CONSERVATION, ENHANCEMENT AND RELEASE FOR LEISURE FISHING

All wild salmonids require cool, high quality water for their juvenile stages. They are very vulnerable to pollution; industries discharging toxic chemicals or effluents such as sewage with a high biological oxygen demand have proved disastrous for many natural stocks. Atlantic and Pacific salmon are anadromous, and therefore require an unobstructed passage from nursery streams through rivers to the sea and back. Even species which complete their life cycle in fresh water often migrate between different parts of the river between nursery, feeding and spawning grounds. The construction of dams for hydroelectric developments and water supply has led to the destruction of several natural populations. The McCloud River hatchery, the origin of the New Zealand quinnat salmon and the rainbow trout introduced to Europe in the nineteenth century, has long been lost, submerged beneath the waters of the Shasta reservoir. Many of the major rivers of Europe such as the Rhine, Elbe and Seine have lost their salmon through a combination of pollution and obstruction.

In some countries the role of culture in sustaining and enhancing natural stocks has been recognized and practical programs have been instituted. The term "salmon ranching" has been applied to the release into the wild of young salmonids at various stages of development in the anticipation that a significant proportion of these will return to their point of release as adults (*see* Chapter 14). Although the term is commonly associated with release of juveniles to increase the return to a trap or fishery, many of same principles apply to release for conservation purposes. Ranching experiments, pilot schemes and full scale projects have been tried for several reasons:

(1) The reintroduction of salmon and trout into rivers where natural stocks have disappeared;
(2) Replacing and enhancing salmon and trout where there has been depletion of natural stocks; and
(3) Producing eggs, fry, parr, smolts or even fully grown fish for release into the wild and subsequent recapture in a commercial or leisure fishery.

### *Reintroduction*

An example of an ongoing project for this purpose is the reintroduction of Atlantic salmon to the River Thames in England. Restocking with hatchery-reared fish follows a major improvement in the quality of the water through reduction of pollution and the building of fish passes at weirs which would otherwise act as barriers to the upstream migration of returning adult salmon.

### *Replacement and Enhancement*

One of the best examples of this practice is in Sweden where natural smolt production has been severely reduced by hydroelectric developments. Dams built across rivers block migration routes and the reservoirs behind the dams often cover large areas of spawning gravel, making fish passes a worthless expense. The natural production of smolts in Sweden was estimated to be 4,000,000 in 1900 and to have fallen to 1,400,000 by 1970, decreasing further since that time (Larssen 1980). At the beginning of the 20th century salmon spawned naturally in more that 80 Swedish rivers; by the 1980s regular spawning occurred in only 13 rivers. Salmon ranching has thus become important for the survival of salmon in Sweden. The first hatcheries were built in the 1860s, at the same time as Stormontfield in Scotland. By the 1880s 4 million eggs were produced every year and released into the wild as fry; survival was therefore probably very low. Improvements in rearing technology and an understanding of factors affecting survival in the wild led to gradual changes in practice; by the 1930s the releases of fry were being supplemented by releases of one-year-old parr. A major breakthrough in the 1950s saw high quality salmon smolts being released from large smolt rearing stations. One of the technological advances which made mass smolt production possible was the development of dry, pelleted diet by the Swedish Salmon Research Institute, financed by the power industry (Bergstrom 1972). There were also major developments in the design of rearing tanks and the optimum pattern of water flow through them. Such advances paved the way for the commercial total culture of salmon (as opposed to ranching which is only partial culture). The number of smolts to be released is determined by law and is currently around 3 million each year.

Even under optimum conditions, survival of hatchery-reared Atlantic salmon in the wild is low, seldom more than 1%. Rearing costs are high as the young fish have to be farmed in fresh water for one or two years before release into the wild as smolts. Thus, the ranching of Atlantic salmon cannot be viewed as being a commercially viable enterprise at present but is justifiable on conservation grounds. The picture is different for some of the species of Pacific salmon, particularly those that migrate to sea very soon after the start of first feeding. This characteristic makes possible the low cost rearing of large numbers of juveniles.

Over the last 20 years great advances have been made in North America in improving the quality of juvenile salmonids released into the wild so that they have a greater chance of survival. In conventional hatcheries, fry were reared on smooth substrates in hatchery trays: survival was poor once these were released. Solomon (1988) listed seven problems associated with such fry which reduced their chance of survival: yolk-sac malformation, deformation of the viscera, liver deformation, fat dystrophy, small size, poor stamina and early requirement for first feeding (i.e., the fish were ready to feed before the natural food supply in the stream was available). Some improvements were made to hatchery design by designing rough substrates for hatching trays. However, greater progress was made with the development of gravel incubation boxes and channels, providing more natural conditions for the incubation of eggs. One example, the Chehalis Hatchery on the Columbia River system in British Columbia has 20 channels, each 24 m long, 2 m wide and 0.2 m deep. These channels have a production capacity of 14 million fry. Fry may be released from the channels immediately after emergence or, preferably, retained for a few days until feeding is well established. Such retention increased the rate of return of pink salmon from the Prince William Sound Aquaculture Corporation farm in Alaska from 2.75% (unfed fry) to 8% when the fry were retained in small-mesh floating cages for two weeks (Solomon 1988).

Huge numbers of juvenile Pacific salmon and steelhead are released each year in North America. In 1987 it was estimated that 1.9 billion juveniles were released from a take of 3.4 billion eggs (Allee 1988). These were divided between states and provinces as shown in Table 2. Juveniles of all five North American Pacific salmon were released, together with steelhead trout. The numbers of fry from the different species are shown in Table 3.

**TABLE 2**

Numbers of juvenile salmonids released around the western Pacific rim in 1987 (from Allee 1988).

| Region | Numbers of juveniles released (millions) |
|---|---|
| Alaska | 840.2 |
| British Columbia | 541.7[a] |
| Washington | 374.1 |
| Idaho | 24.8 |
| Oregon | 90.1 |
| California | 65.8 |
| Total | 1936.7 |

[a] 568 million in 1991 (Anonymous 1991*b*)

### *Egg, Fry, Parr, Smolt and Adult Production*

The releases from the North American and other programs described above obviously produce returns which are exploited by commercial and leisure fishers. However these returns supplement those from natural stocks in most rivers. In other parts of the world, ranched fish dominate catches of returning adult salmon.

In eastern Russia, hatchery production of Pacific salmon dates back to the end of the nineteenth century. The first hatchery was built on Sakhalin in 1912; there are currently 19 hatcheries operating in the Sakhalin region, releasing 600 million juveniles every year. Variations in natural stocks mean that the proportion of hatchery-reared young varies from year to year. For example, the proportion of hatchery-reared pink salmon in the total run has varied between 2 and 68% of the total fry escapement off southeast Sakhalin. The success of this program is reflected in the fact that the catches of adult pink salmon in the Kurilsk Bay area exceed the historical maximum and ranched fish account for as much as 70% of the total harvest (Khorevin et al. 1991). All the chum fry leaving southwestern Sakhalin are hatchery produced.

The most dramatic example of the success of ranching must be in Japan. Hatcheries were established in the nineteenth century and have continued to operate since then. However, following the passing of the Fisheries Resources Protection Act in 1951, salmon propagation was incorporated into national projects and the national Hokkaido Salmon Hatchery. Up to 1970, annual catches of chum salmon were low, below five million fish.

TABLE 3

Numbers (millions) of different species of Pacific salmon released around the western Pacific rim in 1987 (from Allee 1988).

| | **Pink** | **Chum** | **Coho** | **Chinook** | **Sockeye** | **Steelhead** |
|---|---|---|---|---|---|---|
| Alaska | 458.5 | 298.5 | 15.5 | 9.2 | 58.3 | 0.2 |
| B.C. | 16.0 | 199.0 | 22.0 | 55.0 | 247.0 | 2.7 |
| Washington | - | 93.6 | 94.3 | 177.7 | 0.04 | 8.5 |
| Oregon | - | 0.5 | 21.4 | 60.7 | - | 7.5 |
| California | - | - | 1.2 | 58.7 | - | 2.7 |
| Totals | 474.5 | 591.6 | 154.4 | 373.2 | 306.3 | 37.2 |

Many Japanese rivers are now unsuited to natural spawning because of industrial developments; enhancement is therefore essential to maintain stocks and provide fish for the fishery. Since 1971 there has been a virtually continuous increase to around 50 million fish per year. This rise has been achieved through increasing the numbers of juveniles released and improving the quality of each juvenile to increase its chances of survival. Fry releases are now between three and four billion every year. Ocean growth has, however, begun to show a decline (fish are older at return and of a significantly smaller mean size). This indicates that the ocean feeding grounds are possibly now being used to their full capacity and that the numbers of fry released are in excess of the optimum (Khorevin et al. 1991).

Chum salmon are not the only species being released in Japan; figures for releases in 1986 were (in thousands):

| | |
|---|---:|
| chum salmon – | 2,000,458 |
| pink salmon – | 125,638 |
| masu salmon – | 13,795 |
| sockeye salmon – | 75 |

Ranching of Atlantic salmon is far less developed than that of Pacific salmon largely because of the costs of producing juveniles. However in Iceland, returns from sea ranching rose steadily during the 1980s, as shown in Table 4.

The economic importance of the production of salmon and trout for angling has become clear in recent years and can take various forms. Many of the "restocking" programs have as one of their aims (and perhaps the main aim of some supporters!) the increase in the number of fish available for angling. Operators of such programs hope that fish released as juveniles will adopt the behaviour patterns of wild fish, migrating to sea and returning with the wild fish. Another form of salmonid rearing for the table market is that of rearing fish to catchable size and releasing them into a lake or confined river environment where they are recaptured almost immediately by what is termed "put-and-take" fishing. Most of the brown trout reared in the U.K. are for release into this type of fishery; some rainbow trout are also released. On the farm these trout are usually grown at a lower density than the "table" trout to improve their appearance and chance of survival. They may be grown to a larger size than normal table trout. Trials are also taking place into the possibility of put-and-take salmon fisheries where salmon are bought from sea farms and released into still water.

### *Cautionary Note*

Far too frequently, attempts to restock, rehabilitate or enhance salmonid waters have taken place without proper scientific investigation, preparation and monitoring of

**TABLE 4**

Returns of ranched Atlantic salmon in Iceland (OECD).

| Year | Weight of salmon harvested (tonnes) |
|---|---|
| 1982 | 17 |
| 1983 | 32 |
| 1984 | 24 |
| 1985 | 59 |
| 1986 | 65 |

results. The underlying assumption has been that any additional salmon and trout to a system are bound to be beneficial in terms of return to the fishery or increase of the natural spawning stock. Problems to be considered include the state of the existing stock and environment, sources of fish to be stocked (genetics, size and numbers of fish available; *see* Chapter 11), duration of the program and follow-up activities. The Salmon Advisory Committee, a U.K. government-appointed group has identified the following information which should be part of any stocking proposal or program (Anonymous 1991*a*):

- Background:
  - Environmental assessment
  - Stock assessment
  - Details of other work being carried out to remove problems
- Stocking:
  - Source of fish
  - Rearing establishment
  - Number of generations of captive rearing
  - Age of fish to be released
  - Numbers of fish to be released per year
  - Areas of release
  - Methods of release
  - Numbers of years stocking to continue
- Evaluation:
  - Method of evaluation
  - Establishment of benchmarks or controls
  - Duration of the evaluation program

Similar recommendations have been suggested for salmonid stocking in North American waters (Potter and Barton 1986). Where hatchery-reared fish are released to supplement diminished wild stocks, care must be taken that the inevitably increased fishing pressure does not harm the residual wild stocks (*see also* Chapter 14). The strategy suggested for a successful stocking program is set out in Figure 1, taken from the Salmon Advisory Committee report (Anonymous 1991*a*).

## COMMERCIAL CULTURE OF SALMON AND TROUT FOR FOOD

The original salmonid farmers were seeking to produce fish for liberation into the wild. However, it soon became apparent that rainbow trout could be reared to market size on a fish farm.

Rearing of rainbow trout for the table in Europe began in Denmark in the 1890s and by 1914, there were at least 300 farms operating. The major market for the output of these farms was Germany; this vanished with the onset of the First World War and most of the farms closed. The success of the Danish industry was noted in Norway but low winter temperatures prevented the culture of fish in fresh water in the same way as in Denmark. In an attempt to overcome this problem, the Norwegians tried to culture rainbow trout in pens in coastal seawater where winter temperatures were higher. This first recorded seawater pen culture of a salmonid species in 1912 failed because the pens broke up in bad weather. No further serious trials were conducted for more than 40 years when, in the 1950s, the Norwegians began the program of seawater trout and then salmon production which paved the way for the industry as we know it today. The first trout were reared by the Vik brothers at Sykkelven. In the mid-1960s it was appreciated that the techniques being successfully used for rainbow trout could also be used for Atlantic salmon. In 1965, A/S Mowi began Atlantic salmon production in pens; in 1969 the Grontvedt brothers were the first to use cages for the production of Atlantic salmon. The growth of the marine salmon cage farming industry in Norway has been spectacular (Table 5); by 1989 there were 790 farms in operation with a total pen volume of 9.5 million (Bjorndal 1990).

Production of rainbow trout in seawater in Norway has continued, reaching a peak of 5,405 tonnes in 1983, then falling gradually to 3,523 tonnes in 1990. The general belief is that where a site is suitable for the production of both Atlantic salmon and rainbow trout, salmon should be farmed as they will be more profitable.

In 1973 the Norwegian government imposed controls over salmon farming which had a major influence on the pattern of development of the industry. In order to maintain the owner-operator nature of marine salmon farming, a maximum size limit for any farm of 8,000 $m^3$ was imposed; this was increased to 12,000 $m^3$ in 1988. Any farm wishing to increase to the new level must have authorization which will only be given when all the environmental implications of the expansion have been considered. The

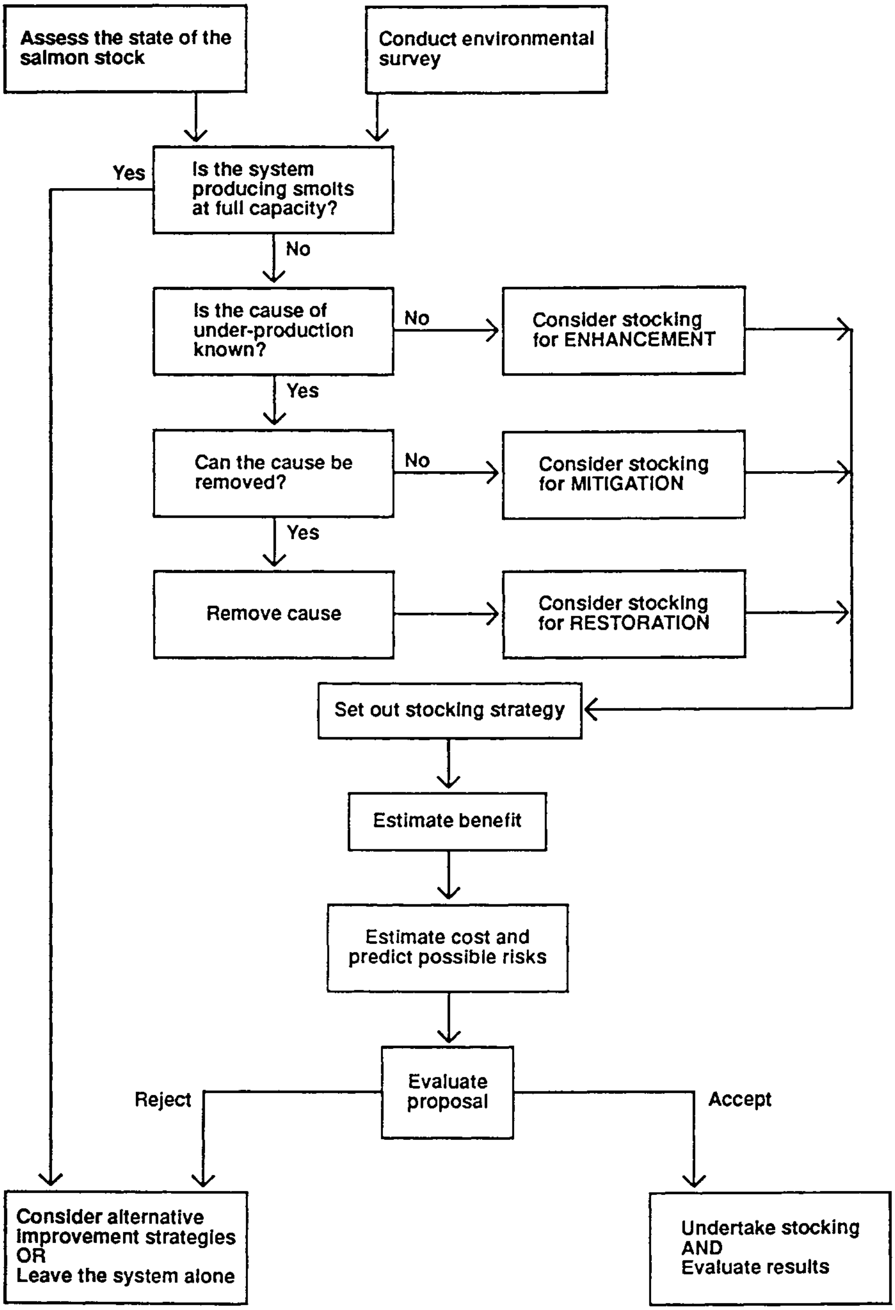
Assess the state of the salmon stock
Conduct environmental survey
Is the system producing smolts at full capacity?
Yes
No
Is the cause of under-production known?
No
Consider stocking for ENHANCEMENT
Yes
Can the cause be removed?
No
Consider stocking for MITIGATION
Yes
Remove cause
Consider stocking for RESTORATION
Set out stocking strategy
Estimate benefit
Estimate cost and predict possible risks
Evaluate proposal
Reject
Accept
Consider alternative improvement strategies OR Leave the system alone
Undertake stocking AND Evaluate results

FIGURE 1. Stocking strategy.

TABLE 5

The production of farmed Atlantic salmon in Norway, 1980-1994.

| Year | Weight of salmon produced (tonnes) |
|---|---|
| 1980 | 4,153 |
| 1981 | 8,422 |
| 1982 | 10,266 |
| 1983 | 17,000 |
| 1984 | 22,000 |
| 1985 | 28,655 |
| 1986 | 45,675 |
| 1987 | 47,417 |
| 1988 | 80,371 |
| 1989 | 114,866 |
| 1990 | 157,944 |
| 1991 | 155,000 |
| 1992 | 141,000 |
| 1993 | 180,000 |
| 1994 | 208,000 |

output of smolt farms is also restricted; individual farms are limited to a maximum output of 1,000,000 smolts.

The development of salmon farming on the west coast and islands of Scotland followed the general pattern of that in Norway, although there have also been differences. There has been a greater involvement of multi-national companies producing hundreds and even thousands of tonnes of salmon, although there also are many small farmers producing 100 tonnes or less annually. There has been a trend to the use of stronger, larger cages to take advantage of the water conditions in less sheltered sites.

In 1990 the weight of the Atlantic salmon produced in countries bordering the Atlantic was almost 50 times the weight caught from the wild by all methods. The growth of farmed output and decline in wild output is shown in Table 6.

Over this period, the commercial fishery for salmon declined. One of the reasons for the decline is the fall in price of salmon because of the increase in the availability of the farmed product. It can thus be argued that the development of salmon farming has reduced the pressure on the wild stocks.

TABLE 6

Farmed Atlantic salmon production and wild salmon catches in the North Atlantic area, 1980 -1990 (ICES figures).

| Year | Salmon output from farms in countries bordering the N. Atlantic (tonnes) | Catches of wild salmon in the N. Atlantic area (tonnes) |
|---|---|---|
| 1980 | 4,783 | 10,081 |
| 1981 | 9,611 | 9,930 |
| 1982 | 12,626 | 8,645 |
| 1983 | 19,972 | 8,732 |
| 1984 | 26,944 | 6,893 |
| 1985 | 37,196 | 8,095 |
| 1986 | 59,393 | 9,249 |
| 1987 | 68,089 | 8,146 |
| 1988 | 111,327 | 7,716 |
| 1989 | 164,722 | 5,894 |
| 1990 | 224,259 | 4,554 |

The output of farmed salmon and trout has continued to rise all over the world where conditions are suitable. Salmon and trout are reared for the table in countries where they are not native and where attempts to introduce them into the wild have been unsuccessful, for example Atlantic and Pacific salmon as well as rainbow trout in Chile and Atlantic salmon in Tasmania.

Most rainbow trout and the early stages of salmon (up to smolting) are reared in fresh water. The simplest rearing system is the traditional, earth pond gravity system, pioneered in Denmark. This low technology system is still widely used for raising rainbow trout to portion-size (200-250 g); improvements in recent years include the incorporation of settling ponds at the downstream end of the farm; which reduce the silt and waste food entering rivers (*see* Chapter 16).

Improvements on the basic earth pond system that allow fish to be kept at higher densities include the lining of the ponds with butyl rubber and aeration or oxygenation by equipment varying in complexity from simple aerators to complex oxygen injection systems. The most advanced freshwater rearing system, used to produce fish of 2 kg or more in weight, is that of concrete lined raceways, long, rectangular tanks through which oxygenated water flows either by gravity or pumping and often with some water recycling. In such systems stocking densities of over 30 kg $m^{-3}$ are achieved. These systems are expensive to build and maintain but may still be economic to operate because of the high density and the rapid growth of rainbow trout, particularly at waters where temperatures are around 15-20°C (*see* Chapter 6).

The freshwater stage of salmon farming usually uses cylindrical tanks made from glass fibre, concrete or galvanized iron, at least for the first few months. The tanks are designed to provide an optimum flow pattern for the young fish with water entering from the side and flowing in a spiral pattern, around the tank and eventually through a central outflow. This ensures that the fish are evenly distributed, preventing crowding damage and allowing even feed distribution which minimizes differences in growth rates between individuals.

At a later stage of development, young salmon (and sometimes trout) may be transferred to cages in freshwater lakes. This practice became necessary when the requirement for running water sites exceeded supply. The cages are small versions of those used in marine farming, usually with a wooden frame because they do not need to withstand such severe conditions as those used at sea. In small lakes with low natural levels of nutrients such as phosphorus, numbers of cages should be restricted and waste feed kept to a minimum to reduce chances of environmental changes, such as blooms of plankton, fuelled by the nutrient increases (Harvey 1988; *see* Chapter 16).

There have been developments in the equipment used for farming fish (*see* Chapter 9) which have made possible the use of more exposed sites and the increase in tonnage. The cages originally used in salmon farming were made from wood with polystyrene flotation and were suitable only for sheltered sites. The use of galvanized steel has allowed the construction of larger, rigid cages of 3000 $m^3$ or more capacity. These cages often incorporate work platforms which are strong enough to carry vehicles and cranes for harvesting. Walkways can be hinged to allow the cages to ride waves better than rigid systems. A different kind of cage suitable for exposed locations is constructed from flexible rubber tubing. This allows fish to be farmed in exposed coastal locations; developments are currently taking place in cage technology which hopefully will allow farming in the open sea (Laird and Needham 1990).

One development in seawater farming that took place in the early 1980s was pump-ashore farming (Laird and Needham 1990). Huge, circular tanks of up to 25 m in diameter were constructed on the shore and fed by pumped seawater. It was felt that the benefits of improved stock control and protection from predators would offset the huge costs involved in pumping. However, in a time of declining salmon prices this has proved generally not to be the case, although there is a special place for such systems in the rearing of broodstock where fish are kept at low density ($<5$ kg $m^{-3}$) and light levels can be controlled to alter the timing of sexual maturity. Pump-ashore systems have not become part of the mainstream of salmon farming.

The developments that have taken place in cage technology have been accompanied by developments to other types of equipment needed in fish farming.

Because of the need to handle larger quantities of fish than in the original cages (these could hold a maximum of 5 tonnes, the cages now used can hold 30 tonnes or more), fish-handling equipment has been developed. Cranes mounted on the cages or on boats are used for lifting nets and harvest bins. One of the latest developments is the use of fish pumps to transport live fish between cages or to harvest tables. These pumps minimise scale damage and stress, reducing the risk of disease associated with transfer and grading operations. There have been developments in feed handling and distribution. Many farmers stopped using automatic feeding devices and reverted to hand feeding because of the better opportunity for stock observation, but there is an increase in the use of automated blower systems to deliver the quantities of feed required for fish in large volume cages.

Equipment has been developed to deal with some of the problems encountered on and around fish farms. Nets hanging into water quickly become covered in fouling seaweeds, barnacles and molluscs. Toxic paints such as tributyl tin were used to counteract this; it is now illegal to use this chemical so new methods have been found. These include net-washers and hoses which remove fouling organisms without using chemicals. Another problem on many cage farms is that of damage to nets and fish caused by predators such as birds (herons, cormorants) and mammals (seals). Seals, which can cause so much damage to a cage that the whole stock of salmon is lost, are particularly difficult to deal with. Ultrasonic scaring devices have been developed and have had mixed success in preventing seal attacks. Anti-predator nets are now attached above cages and tanks to prevent access by birds and around individual or groups of sea cages to keep seals away from stock. If correctly set, these nets are usually effective. New developments include devices for tensioning underwater cage nets; it is a characteristic of the industry that this method has been developed by farmers and trials have been carried out on operating farms rather than in research stations. A novel seal deterrent introduced in 1994 is a glass fibre killer whale, moored below the surface near cage groups: this has had some initial success.

### *Worldwide Farm Production of Salmon and Trout*

Table 7 shows the 1989 (and 1994 where available) figures for the output of salmon and trout in the major producing countries throughout the world. Most of the production listed under trout is of rainbow trout or steelhead, however smaller quantities of brown trout and brook trout (*Salvelinus fontinalis*) may be included in the figures. Information has been gathered from various sources, including the OECD and the European Salmonid Federation.

TABLE 7

Production of salmon and trout (tonnes) in major producing countries. Figures given are for 1989 production except where indicated and 1994 estimates.

| Country | | Species | 1989 production | 1994 estimates |
|---|---|---|---|---|
| Australia | (Tasmania) [a,b] | Atlantic salmon | 3,000 | 2,600 |
| | (Tasmania) | seawater rainbow trout | 600 | |
| | (mainland) | freshwater trout | 1,000 | |
| Austria | | trout | 3,500 | |
| Belgium | | trout | 800 | |
| Canada | [a] (east) | Atlantic salmon | 7,810 | 15,000[c] |
| | [a] (west) | Atlantic & Pacific salmon | 13,500 | |
| | | trout | 4,000 | |
| Chile [a] | | salmon | 19,406 | 30,000 |
| [a] | | trout | 4,281 | |
| China | | trout | 700 | |
| Denmark | | trout | 31,700 | |
| Faroes [a] | | Atlantic salmon | 13,000 | 20,000 |
| [a] | | trout | 2,000 | |
| Finland [d] | | trout | 4,000 | |
| France [a] | | salmon | 150 | 500 |
| | | trout | 35,850 | |
| Germany (combined) | | trout | 25,590 | |
| Greece | | trout | 2,000 | |
| Iceland [a,e] | | salmon | 2,800 | 2,000 |
| | | trout | 700 | |
| Ireland [a] | | salmon | 8,175 | 14,000 |
| | | trout | 1,029 | |
| Italy | | trout | 31,000 | |
| Japan [a] | | salmon | 20,000 | |
| Japan [a] | | trout | 15,600 | |
| Netherlands | | trout | 220 | |
| New Zealand [f] | | salmon | 611 | |
| Norway [a] | | salmon | 157,944 | 208,000 |
| Norway [a] | | trout | 3,523 | |
| Portugal | | trout | 1,200 | |
| South Korea | | trout | 1,200 | |
| Spain [a] | | salmon | 150 | 1,000 |
| | | trout | 16,000 | |
| Sweden [g] | | salmon | 224 | 400 |
| | | trout | 2,800 | |
| Turkey [f] | | trout | 990 | |

**TABLE 7 cont'd.**

| Country | Species | 1989 production | 1994 estimates |
|---|---|---|---|
| United Kingdom [a] | salmon | 32,350 | |
| [h] | salmon | 41,000 | 64,000 |
| | trout | 15,000 | |
| U.S.A. [a] (east) | salmon | 2,250 | 12,000[i] |
| [a] (west) | salmon | 1,250 | |
| | trout | 26,000 | |
| U.S.S.R. [a] | salmon | 3,000 | |
| | trout | 6,000 | |

[a] 1990 production
[b] Source Forteath (pers. comm. 1991)
[c] Atlantic salmon, east and west combined
[d] Over 2,000,000 salmon smolts are produced every year
[e] In addition, 65 tonnes of Atlantic salmon were produced by sea ranching in 1989
[f] 1986 production
[g] 1987 production
[h] 1991 production
[i] east and west combined

## THE FUTURE

The growth of Atlantic salmon farming in the main producing countries through the 1980s has been dramatic. However, several problems have arisen at the beginning of the 1990s to halt the growth of the industry and even put it in decline in some of the major producing countries. Such problems include disease, economics and adverse environmental effects.

### *Disease*

Diseases such as furunculosis caused by the bacterium *Aeromonas salmonicida* have, in the past, caused massive mortalities on Scottish salmon farms. Some strains of the bacteria have become resistant to the antibiotics licensed for use. However, the successful development and use of vaccines combined with site fallowing have brought about a major reduction in the impact of this disease on fish survival and also eliminated the use of antibiotics on most farms. Vaccines for other diseases such as *Vibrio* and enteric red mouth (ERM) are also effective (*see* Chapter 13).

Parasitic diseases are also causing major problems in the salmon farming industry. Copepod ectoparasites, the salmon louse *Lepeophtheirus salmonis* and the fish louse *Caligus elongatus*, have been responsible for massive mortalities. The treatment devised when these parasites first became a problem involves dosing whole cages with a solution based on the organophosphorus compound, dichlorvos. Although effective, this compound is dangerous to human health if incorrectly used and has been a focus for those concerned with the environmental implications of salmon farming as, following treatment, it is released into the environment (*see* Chapter 16). The chemical is on the "red list" and, in the U.K., used under an annually renewable licence. It is unlikely that this licence will continue to be issued for much longer. The lack of measures for disease prevention and control is a symptom of the rapid expansion of the industry over a very short period of time; there has been neither the time nor the resources for the research and pilot schemes necessary. Improvements in husbandry through site-fallowing and the isolation of year classes have reduced the problem but not eliminated it. New measures currently being developed in the hope of controlling sea lice include the use of wrasse which act as cleaner fish to remove lice from the salmon. However, even if wrasse are shown to be effective at controlling sea lice, availability of wrasse, their culture and their own disease problems are likely to be limiting factors for some time. In the meantime, other chemicals such as pyrethrum-types, Ivermectin and hydrogen peroxide are undergoing trials.

### *Economics*

Between 1980 and 1990 the production of farmed salmon in the north Atlantic area rose from under 5,000 tonnes per annum to 224,000 tonnes per annum. This has obviously had a major effect on the market; prices have fallen as successive marketing campaigns have failed to increase demand for the product in pace with the increase in production. Even though there have been these massive production increases there are still problems with seasonal supply of fish of a desired size and quality. Also, the problems of premature sexual maturation as jacks or grilse have still not been fully solved. Developments in the production of out-of-season smolts are helping to even out seasonal inbalances in supply and demand of salmon of different size categories (*see* Chapter 8). For salmon farming and the intensive production of trout to continue to grow and prosper, new markets and products must be developed and sustained (*see* Chapter 15).

### *Environmental Effects*

The 1980s saw a major increase in the amount of interest and attention focused on the care of the natural environment and the humane treatment of stock on farms. This has coincided with the growth of salmon farming and, as salmon and trout have high water quality requirements and farms are often situated in areas of outstanding beauty, there have often been conflicts. Most farmers are learning the benefits of environmental awareness, improving the appearance of their farms and considering how to farm fish in a more sustainable fashion.

The importance of water quality has always been known but more information, for example on sites that may be susceptible to toxic algal blooms, is becoming available. Farmers are also becoming aware of measures which they themselves can take to improve water quality and the quality of the stock on the farm. These include the following:

*Siting.* Because of developments in equipment technology it is no longer necessary to site cage farms in the extremely sheltered bays and inlets favoured at the start of cage farming. More exposed sites have stronger tidal currents which ensure that wastes are dispersed away from the cages and minimize the build-up of sediment underneath cages (*see* Chapter 16).

*Stock.* It is obviously important to start the farming process with disease-free stock. Most countries now have facilities for testing eggs and broodfish to ensure their health status. Research is helping in the identification of strains of salmonids which have favoured characteristics for growth at particular sites (e.g., disease resistance, growth rate, acid tolerance, age at maturity, egg size) and controlled breeding programs have been established (*see* Chapters 4 and 11).

*Feeding.* Developments in feed technology have improved the quality of the feed and made possible increases in food conversion efficiency. Progress is being made in the production of low pollution diets; coupled with improved feeding practices, these have led to a reduction in waste and the pollution of the environment (*see* Chapters 9 and 10).

*Husbandry.* Intensive salmon and trout farmers are fast learning the lessons that land farmers have had many thousands of years to assimilate. The importance of good husbandry applies on land and in water. Salmonid farmers are learning that reducing stocking densities below those which are theoretically possible leads to healthier stock. The principle of site-fallowing is also being followed to good effect; where possible, cage salmon farmers should have access to at least three sites, one of which is unused at any time. Stocking of cage groups with one year class at a time prevents cross-infection. Where more than one operator is farming in the same area, management agreements are being drawn up to cooperate over stocking and harvesting policies.

The salmon and trout farming industry is facing a difficult time at present and probably for a few years to come. However, new techniques worked out on the farm and in research facilities should help to ensure its long-term survival, as an important part of the rural economy of many countries throughout the world.

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## Chapter 2

# GENERAL BIOLOGY OF SALMONIDS

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## INTRODUCTION

The salmonid group is one of the most widely and intensively studied fish groups and published literature is available on practically every facet of their biology. To condense the many fascinating aspects of the biology of salmonid fishes into one brief chapter would be impossible and unjust. This chapter is meant to provide an introduction and background to various features of salmonid biology at the organism level discussed in the following more comprehensive chapters. The organismal biology of salmonids is presented very generally and much of the information pertains to most teleost fishes.

Traditionally, the salmonid fishes have been very popular in commercial fisheries and are highly prized for sport. Most species have anadromous forms, which make them amenable to culture in both freshwater and marine environments. Many species of salmonids have been transplanted into waters far beyond their native ranges throughout the world for both recreational and commercial purposes (*see* Chapter 1). For example, rainbow and brown trout have been introduced successfully in places as unlikely as Madagascar and Hawaii (rainbow trout), and the Falkland Islands (brown trout) (MacCrimmon and Marshall 1968; MacCrimmon 1971; Maciolek 1984).

The salmonid fishes discussed in this chapter include those of the genera: *Oncorhynchus*, *Salmo*, *Salvelinus*, and *Hucho*. Strictly speaking, the salmonids of interest for aquaculture are those of the subfamily Salmoninae, as the family Salmonidae also includes the whitefishes (Coregoninae) and graylings (Thymallinae) (Nelson 1994).

Many species of salmonids exist world-wide (Table 1), but as they are "coldwater" fishes their native ranges are restricted generally to temperate waters. Additional salmonid genera, not discussed herein, include *Brachymystax* and *Salmothymus* (Nelson 1994), and *Salvethymus*, a possible ancestral form discovered recently in northeastern Russia (Chereshnev and Skopets 1990).

The salmonids may be considered a phyletically "primitive" group as they lack the high degree of specialization evident in "higher" teleost groups, for example, the percomorph fishes (Moyle and Cech 1988). The salmonids may also be considered as a relatively "plastic" group and, within a species, a number of distinct genetic races or stocks may be evident (e.g., *see* Simon and Larkin 1972; Billingsley 1981; Gresswell 1988). As a result, nomenclature has been somewhat problematic and a number of name changes have been made in the past few decades. Recently, native North American trouts of the genus *Salmo* were included with the Pacific salmons (Smith and Stearley 1989; Stearley and Smith 1993; *see* Robins et al. 1991*a*) and the once familiar scientific name *Salmo gairdneri* for rainbow trout is now only of historical interest.

This chapter is intended to introduce the reader to the general anatomy, environmental requirements, physiological processes, bioenergetics, and reproductive characteristics of salmonid fishes. Much of the material in this chapter has been summarized from a number of compilations on fish biology (e.g., Lagler et al. 1977; Bond 1979; Bone and Marshall 1982; Cailliet et al. 1986; Moyle and Cech 1988) and the excellent review chapters provided in the Fish Physiology series edited by W.S. Hoar, D.J. Randall, and others (pertinent chapters are cited throughout). Readers who wish to delve further into the natural history and biology of salmonids and their physiological processes should consult those sources and other species-specific literature, many of which are listed in Table 2. Numerous citations to general texts and reviews rather than primary studies have been made to provide adequate source material for future reference.

## STRUCTURAL AND FUNCTIONAL ANATOMY

### *External Morphology*

Although size may vary with species, stock, and environmental conditions, all members of the subfamily Salmoninae are remarkably similar in their external appearance and are generally fusiform in shape. The integument is covered with small cycloid scales, the numbers of which along specific vectors are used for species separation (e.g., pored lateral line scales; Table 3).

TABLE 1

Scientific name, familiar common names, and continents of native distribution of important salmonid fishes of the world. Scientific nomenclature is that currently accepted by the American Fisheries Society (Robins et al. 1991*a*, 1991*b*) except for amago salmon (Kato 1991).

| Scientific name | Common names | Native continents |
|---|---|---|
| ***Oncorhynchus*** **spp.** | **Pacific Salmon and Trouts** | |
| *O. aguabonita* | golden trout | N. America |
| *O. apache* | Apache trout | N. America |
| *O. chrysogaster* | Mexican golden trout | N. America |
| *O. clarki* | cutthroat trout | N. America |
| *O. gilae* | Gila trout | N. America |
| *O. gorbuscha* | pink salmon, humpback | Asia, N. America |
| *O. keta* | chum salmon, dog salmon | Asia, N. America |
| *O. kisutch* | coho salmon, silver salmon | Asia, N. America |
| *O. masou* | masu salmon, cherry salmon, yamame | Asia |
| *O. mykiss* | rainbow trout, steelhead, Kamchatkan trout | Asia, N. America |
| *O. nerka* | sockeye salmon, kokanee, red salmon | Asia, N. America |
| *O. rhodurus*[a] | amago salmon, biwamasu | Asia |
| *O. tshawytscha* | chinook salmon, king salmon, spring salmon, tyee | Asia, N. America |
| ***Salmo*** **spp.** | **Trouts** | |
| *S. ischchan* | Sevan trout | Europe |
| *S. letnica* | Ohrid trout | Europe |
| *S. platycephalus* | flathead trout, ala balik | Europe |
| *S. salar* | Atlantic salmon, Sebago salmon, ouananiche | Europe, N. America |
| *S. trutta* | brown trout, sea trout | Europe |
| ***Salvelinus*** **spp.** | **Charrs (Chars[b])** | |
| *S. alpinus* | Arctic charr, char, Sunapee trout | Asia, Europe, N. America |
| *S. confluentus* | bull trout, bull charr | N. America |
| *S. fontinalis* | brook trout, brook charr, speckled trout | N. America |
| *S. leucomaenis* | whitespotted charr, Japanese charr | Asia |
| *S. malma* | Dolly Varden charr | Asia, N.America |

TABLE 1 cont'd.

| Scientific name | Common names | Native continents |
|---|---|---|
| ***Salvelinus* spp. cont'd.** | **Charrs (Chars)** | |
| *S. namaycush* | lake trout, lake charr, mackinaw | N. America |
| ***Hucho* spp.** | **Huchens** | |
| *H. hucho* | huchen, Danube salmon | Europe |
| *H. perryi* | Japanese huchen, stringfish | Asia |
| *H. taimen* | taimen | Asia, Europe |
| ***Salmothymus* spp.** | | |
| *S. obtusirostris* | Adriatic trout, mekousna | Europe |
| **Hybrids** | | |
| *Salmo trutta* X *Salvelinus fontinalis* | tiger trout | |
| *Salvelinus fontinalis* X *S. namaycush* | splake | |

[a] considered same species as *O. masou* by Robins et al. (1991*b*)

[b] Many prefer the use of 'charr' or 'char' instead of 'trout' in the common names of some *Salvelinus* species (e.g., lake charr); both spellings are acceptable.

Body colouration in marine and lacustrine forms is usually silvery, whereas freshwater juveniles and strictly riverine and some lacustrine forms are generally dark-backed without the intense silvering. Body spotting is variable and is used as a gross characteristic to separate species. However, body spotting is not reliable for speciation because of significant stock and environmental effects. As a general rule, dark-spotted salmonids include *Oncorhynchus* and *Salmo*, whereas light-spotted salmonids include *Salvelinus*.

Fin placement is consistent among species and genera. Salmonids are soft-rayed fishes and possess a single soft-rayed dorsal and anal fin, a homocercal caudal fin (tail), and paired pectoral and pelvic fins (Figure 1). The pelvic fins are located abdominally and are accompanied by a small axillary process that projects posteriorly from the fin's anterior insertion point. Salmonids are one of the few fish groups that have an adipose fin, located along the dorsal midline posterior to the dorsal fin ahead of the tail. Fishes of the genus *Hucho* have a characteristically large adipose fin compared with other

TABLE 2

Selected references, listed alphabetically, on the biology, systematics, and natural history of salmonid species.

| **Reference** | **Annotation** |
|---|---|
| Allen and Hassler (1986) | Life history, environmental requirements, and management summary of chinook salmon |
| Balon (1980) | Major text containing comprehensive chapters on the biology of the important species and hybrids of the genus *Salvelinus* |
| Barnhart (1986) | Life history, environmental requirements, and management summary of steelhead |
| Behnke (1992) | Extensive monograph on the systematics and biology of native western North American trout species |
| Bjornn and Reiser (1991) | Summary review of natural habitat requirements for many North American salmonids |
| Bonar et al. (1989) | Life history, environmental requirements, and management summary of pink salmon |
| Childerhouse and Trim (1979) | Semi-popular but relatively comprehensive treatise on North American west coast salmonids |
| Foerster (1968) | A thorough account of the life history, ecology, and fisheries of the sockeye salmon |
| Frost and Brown (1967) | Dated but informative account of the biology and life history characteristics of brown trout in Great Britain |
| Gall (1992) | Symposium proceedings on physiology, genetics, nutrition, husbandry and economics of rainbow trout in aquaculture context |
| Goetz (1989) | Review of the zoogeography and biology of the bull trout in the North American Pacific Northwest |
| Gresswell (1988) | Collection of papers describing the present status of interior stocks of cutthroat trout in North America |

**TABLE 2 cont'd.**

| Reference | Annotation |
|---|---|
| Groot and Margolis (1991) | Comprehensive up-to-date accounts of the life histories and many ecological aspects of all of the Pacific salmons by separate authors |
| Groot et al. (1995) | Comprehensive synthesis of the physiological functions and adaptations of Pacific salmon |
| Hale et al. (1985) | Summary review of the life history, biology, and habitat requirements of chum salmon |
| Havey and Warner (1970) | Comprehensive account of landlocked Atlantic salmon in Maine |
| Heen et al. (1993) | Text includes geographic distribution of salmon aquaculture, environmental concerns, and salmon farm management and economics. |
| Hickman and Raleigh (1982) | Summary review of the life history, biology, and habitat requirements of cutthroat trout |
| Holčík et al. (1988) | Comprehensive synthesis of the life history, biology, management, and culture of the huchen and other species of the genus *Hucho* |
| Jobling et al. (1993) | Review of feeding, growth, and environmental requirements of Arctic charr |
| Johnson and Burns (1984) | Symposium proceedings on taxonomy, biology, life history, and management of Arctic charr |
| Kawanabe et al. (1989) | Symposium proceedings on genetics, physiology, behaviour, life history, population ecology, and management of various charrs and masu salmon |
| Laufle et al. (1986) | Life history, environmental requirements, and management summary of coho salmon |
| MacCrimmon and Gots (1972) | Comprehensive summary of the stocking history and present distribution of rainbow trout populations in the Great Lakes |

**TABLE 2 cont'd.**

| Reference | Annotation |
|---|---|
| Marcus et al. (1984) | Summary review of the life history, biology, and habitat requirements of lake trout |
| McMahon (1983) | Summary review of the life history, biology, and habitat requirements of coho salmon |
| Meehan and Bjornn (1991) | Summary review of the distribution, life history, and selected aspects of the ecology of North American salmonids |
| Meerburg (1986) | Proceedings of a workshop on factors affecting salmon age and size of maturity, primarily Atlantic salmon |
| Mills (1986) | Short synopsis of the biology of Atlantic salmon in Scotland |
| Mills (1989) | Comprehensive account of Atlantic salmon ecology and management, with emphasis on the latter |
| Neave (1958) | Discussion paper on the origin and speciation of the genus *Oncorhynchus* |
| Pauley et al. (1986) | Life history, environmental requirements, and management summary of steelhead |
| Pauley et al. (1989*a*) | Life history, environmental requirements, and management summary of sea-run cutthroat trout |
| Pauley et al. (1989*b*) | Life history, environmental requirements, and management summary of sockeye salmon |
| Pearcy (1992) | Synthesis on the ecology of the ocean phase of northeastern Pacific salmon with emphasis on coho salmon |
| Power (1969) | Comprehensive study of the life histories of Atlantic salmon stocks in Ungava Bay, Canada |
| Raleigh (1982) | Summary review of the life history, biology, and habitat requirements of brook trout |

**TABLE 2 cont'd.**

| Reference | Annotation |
|---|---|
| Raleigh and Nelson (1985) | Summary review of the life history, biology, and habitat requirements of pink salmon |
| Raleigh et al. (1984) | Summary review of the life history, biology, and habitat requirements of rainbow trout |
| Raleigh et al. (1986*a*) | Summary review of the life history, biology, and habitat requirements of chinook salmon |
| Raleigh et al. (1986*b*) | Summary review of the life history, biology, and habitat requirements of brown trout |
| Shearer (1992) | Account of the life history, exploitation, and management of Atlantic salmon |
| Smith and Carter (1973) | Some papers included on physiology and smoltification in Atlantic salmon |
| Smith et al. (1987) | Limited number of specific papers on the biology of sockeye salmon |
| Stefanovic (1966) | Comprehensive account of the racial characteristics, growth, and food habits of the Ohrid salmonids, particularly Ohrid trout |
| Stickney (1991) | Text includes life history synopses of major salmon and trout species and policy issues associated with their culture |
| Tanaka (1965) | Review of the biology, offshore distribution, and fisheries of masu salmon |
| Trotter (1987) | Biological summary of the major subspecies of cutthroat trout in North America |
| Wilimovsky (1962) | Symposium proceedings on the ecology of pink salmon populations |
| Willers (1991) | Semi-popular account of the life history and biology of the major salmonids |

**TABLE 3**

Examples of selected meristic features in important salmonids (Berg 1948; Muus 1971; Scott and Crossman 1973) (for example only, as many features are variable; other sources will often cite slightly different values [e.g., Wheeler 1969; McPhail and Lindsey 1970; Hart 1973; Scott and Scott 1988]).

| Genus and species | Gill rakers | Principal dorsal fin rays | Pored lateral line scales | Pyloric caeca |
|---|---|---|---|---|
| ***Oncorhynchus*** | | | | |
| pink salmon | 24–35 | 10–15 | 147–205 | 95–224 |
| chum salmon | 11–17 | 10–14 | 124–153 | 140–249 |
| coho salmon | 18–25 | 9–12 | 121–148 | 45–114 |
| masu salmon | 18–22 | 13–18[a] | 130–140 | 35–76 |
| sockeye salmon | 29–43 | 11–16 | 120–150 | 45–115 |
| chinook salmon | 16–26 | 10–14 | 130–165 | 90–240 |
| cutthroat trout | 14–22 | 8–11 | 116–230 | 27–57 |
| rainbow trout | 16–22 | 10–12 | 100–150 | 27–80 |
| ***Salmo*** | | | | |
| Atlantic salmon | 15–20 | 10–12 | 109–121 | 40–74 |
| brown trout | 14–17 | 12–14 | 120–130 | 30–60 |
| ***Salvelinus*** | | | | |
| Arctic charr | 19–32 | 10–12 | 123–152 | 20–74 |
| brook trout | 14–22 | 10–14 | 110–130 | 23–55 |
| Dolly Varden | 11–26 | 10–12 | 105–142 | 13–47 |
| lake trout | 16–26 | 8–10 | 116–138 | 93–208 |
| ***Hucho*** | | | | |
| huchen | 16 | 12–14[a] | 180–220 | ≈200 |

[a] all dorsal rays

salmonids (Holčík et al. 1988). The purpose of the adipose fin is not precisely known, but it may have a passive hydrodynamic function in juveniles (Lindsey 1978).

The differences among species in fin-ray counts, fin length, tail shape, etc., are useful parameters for biologists for species identification (e.g., Table 3).

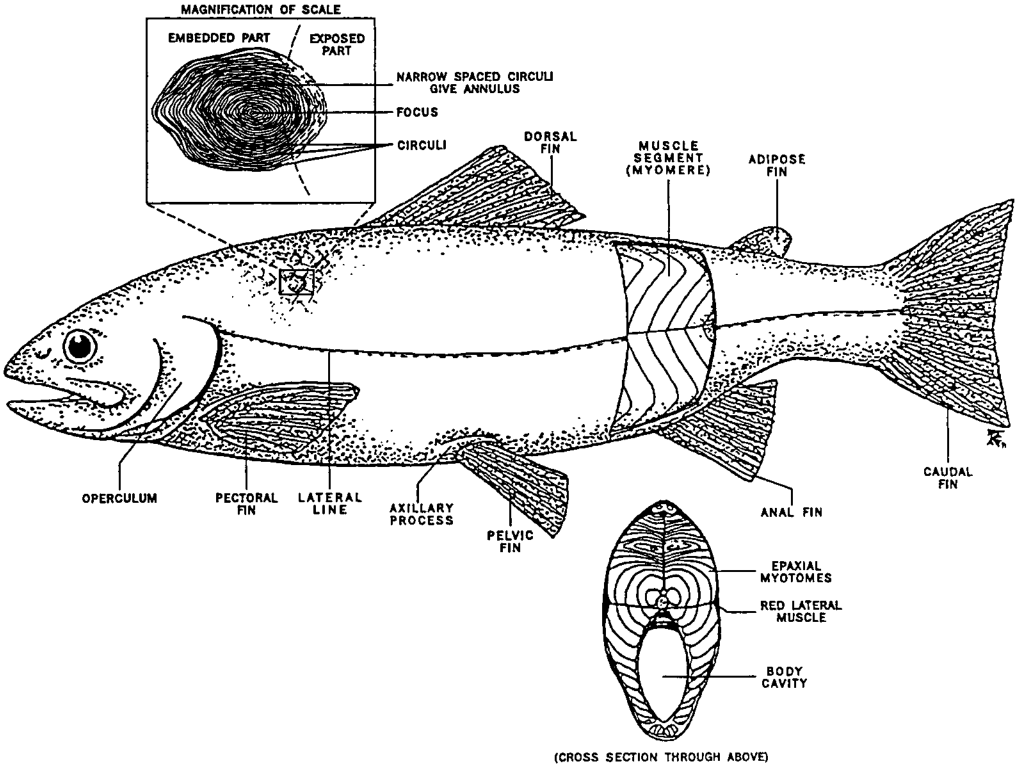

FIGURE 1. External appearance of a typical salmonid fish showing fin placement and cutaway section to illustrate myotomes. Insets show a typical salmonid cycloid scale and a cross section through the fish illustrating muscle pattern (after Cailliet et al. 1986).

### *Internal Structure*

*Skeleton.* The skeleton, which is for support and muscle attachment, has been studied extensively, primarily for systematic reasons. In particular, Vladykov (1962) examined the skull and caudal skeleton in great detail to provide a means of determining racial origin of ocean-caught Pacific salmon. Hikita (1962) studied skeletal anatomy as well as the general external features of different species of the genus *Oncorhynchus* in a taxonomic context.

*Musculature.* The detailed musculature of salmonids is not well described, but comprehensive reviews of teleost musculature and their physiology are presented by Winterbottom (1974) and Bone (1978). Forward motion results from a combination of the backward passage of transverse waves along the body and lateral motion of the caudal fin (Bone and Marshall 1982). Both actions result from the systematic operation of the myomeres, or W-shaped muscle fibre segments of the large axial muscles (Figure 1).

Muscle, most of which is white trunk muscle, comprises about 56 to 66% of salmonid body mass (Smith 1982; Satchell 1991). White muscle is poorly vascularized (whence the name) and functions anaerobically. This muscle tissue is used in burst swimming activity when such action is required for rapid predator avoidance or prey capture. Salmonids can only maintain burst swimming activity [e.g., >2 - 10 body lengths (BL)/s] for short durations (e.g., <20 s; Beamish 1978), after which they require a period of recovery so that accumulated lactic acid in the muscle tissue can be eliminated. The burst response of salmonids is extremely rapid and is controlled sympathetically by the Mauthnerian system (Diamond 1971; Lindsey 1978). This system consists of two nerve cells (Mauthner cells) located in the medulla of the brain and connected to two long axons that run the length of the spinal cord. The Mauthner cells alternately stimulate white muscle on each side of the body to constrict and relax opposite to each other, allowing the rapid undulating motion characteristic of the burst or startle response.

The important but less extensive red muscle is used for routine activity and sustained swimming, particularly for extended time periods. For example, the estimated sustained cruising speed of Pacific salmon is about 0.8 to 1.5 BL/s but may be up to 2 BL/s (Lindsey 1978). Red muscle may only be about 1% of the body mass (Satchell 1991) but the proportion of red to white muscle increases toward the posterior of the fish. Red muscle may comprise up to 15% of a salmonid's muscle mass in the caudal region (Love 1980). Red muscle, which functions aerobically, is well vascularized and, hence, well oxygenated.

*Internal Organ Placement.* Characteristic of most teleosts, the heart is located well forward in the body cavity (Figure 2). The kidney in salmonids is located dorsally in the visceral cavity, lying ventrally against the vertebral column (Figure 2). The gas or swim bladder is located ventral to the kidney. Much of the body cavity in immature fish is taken up by the gut, which consists of the esophagus, the glandular cardiac portion and

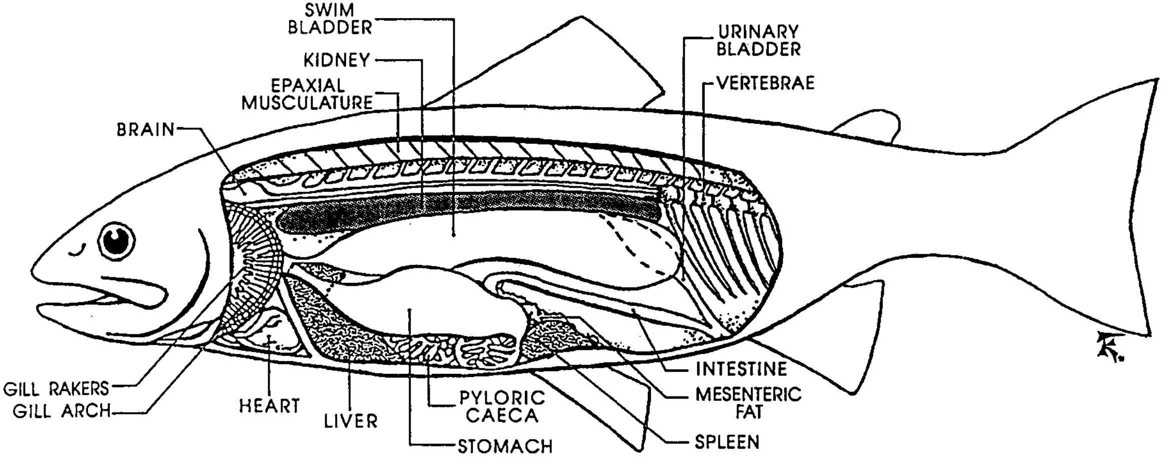

FIGURE 2. Lateral cutaway view of a typical salmonid fish to show major anatomical features and their location (after Smith and Bell 1976; Cailliet et al. 1986).

the muscular pyloric portion of the stomach, the pyloric caeca, and ascending and descending limbs of the intestine.

Whitish fat deposits usually accumulate in strips in the mesentery tissue and abdominal space surrounding the gut, particularly around the pyloric caeca in salmonids. In well-fed hatchery fish, for example, mesenteric, or visceral, fat deposits may cover caeca completely and the extent of deposition may be used to assess the energy reserve status of young salmonids (Goede and Barton 1990). Dorsal and lateral to the gut are the gonads and in fully mature fish, these organs may dominate the visceral cavity.

Smith and Bell (1976) provided a useful summary of anatomical features of Pacific salmon along with clear diagrams indicating organ placement. For more general or comparative descriptions of teleost anatomy, one may refer to Cailliet et al. (1986).

### *Organs and Systems*

*Gills and Related Structures.* The functions of the gills are to: take in oxygen ($O_2$), eliminate waste, and regulate hydromineral balance. Salmonids have four pairs of gill arches. Four gill arches, one from each pair, are located in a common opening on each side of the head and covered by an operculum. Multiple pairs of gill filaments are anchored to each gill arch and numerous plate-like secondary lamellae are attached to each filament. The primary site of gas exchange in salmonids is across the lamellar surface. Within each lamella are specialized cells for gas exchange, afferent vessels to the site of gas exchange, and efferent vessels leading from the site of gas exchange (*see* Respiration). Detailed descriptions and diagrams of fish gill anatomy and function may be found in Randall (1970), Hughes (1984), and Moyle and Cech (1988), among others.

Associated with each gill arch is a series of gill rakers, obvious as projections on the opposite side of the arch from the gill. Gill rakers serve to protect the gill from large debris and to trap food, particularly plankton. Numbers of gill rakers vary among species and may serve as a meristic feature to aid in species identification (Table 3) although gill raker numbers can be affected by environmental conditions as well.

Salmonids also have a rudimentary first "gill" known as the pseudobranch, located on the underside of the operculum and visible as a distinct reddish area because it is highly vascularized. The function of the pseudobranch is not clear but anatomical evidence suggests that the pseudobranch may serve as an oxygen sensor, thereby influencing blood flow in the gills, or may be associated with maintaining oxygen to the choroid gland behind the eye (Smith and Bell 1976). The pseudobranch is rich in carbonic anhydrase and may function to decrease possibly harmful $CO_2$ levels in arterial blood before it reaches the choroid (Nicol 1989). However, Laurent and Dunel-Erb (1984) concluded that evidence suggesting the variety of pseudobranch functions is questionable and indicated that the role of the pseudobranch is a partly sensory one (i.e., involved in baro- and chemoreception).

*Heart and Circulation.* Five components make up the primary or cardiovascular circulatory system: the heart, arterial system, capillary system, venous system, and the blood.

*1). Heart.* The thin pericardium of the heart surrounds a relatively large, thin-walled atrium and a thick-walled ventricle. Venous blood enters the atrium via another thin-walled chamber, the sinus venosus, which acts to collect the blood. Assisted by the action of the skeletal muscles, the heart fills by slight venous pressure. When full, the atrium contracts and blood enters the muscular ventricle. Ventricular pumping forces blood into the arterial system through the bulbus arteriosis. The bulbus arteriosis has relatively elastic walls that act to dampen pressure extremes and "smooth out" blood flow into the arterial system. The heart is an automatically contractile organ and contraction is initiated by stimulation of "pace-maker" cells located, based mostly on anatomical evidence, in the vicinity of the junction of the sinus venosus and atrium (sinoatrial canal) (Satchell 1991).

The heart is considered an "excitable" tissue; that is, it contracts by stimulation. Stimulation of heart muscle is adrenergic via the sympathetic nervous system whereas relaxation is cholinergic or parasympathetic. Heart rate and stroke volume are influenced by changes in temperature and blood volume, which change pressure. Increased filling volume will produce an increased ventricular contraction, the main limiting factor being the stretch resistance of the muscle.

*2). Circulation.* Circulation of blood is initially into the arterial system, which is a rigid, high pressure network. Blood enters the compliant, low pressure venous system through the capillary network. Within the peripheral tissues, gases, nutrients, metabolites, hormones, and other materials are exchanged across the capillary membranes. The capillary network also controls peripheral resistance of the circulatory system and, thus, blood flow. Smith and Bell (1976) provide photographs of casts and diagrams of the circulatory system that clearly illustrate the circulation and the vascular pattern of salmonids.

The "physics" of blood flow may be considered by the equation:

$$F = \Delta P/R \tag{1}$$

where F = flow, Δ P = pressure difference along the length of a vessel, and R = resistance (Satchell 1991). Pressure may be changed by altering the heart rate or stroke volume and resistance will vary with the length of the vessel, viscosity of the blood, and cross-sectional area of the system. As length and viscosity are essentially constant, control of the cross-sectional area of the vessels exerts the major influence on resistance and, therefore, blood flow. Control of peripheral resistance takes place in the capillary network where constriction and relaxation of vessels is controlled by nerves or hormones.

*Blood.* Blood volume in primary circulation ranges between about 3 to 7% (i.e., ml/100 g) in salmonids (Smith 1982) and is comprised of cells and plasma. Blood cell formation takes place primarily in the spleen and head kidney (Satchell 1991). The thymus tissue, located in the pharyngeal area behind the opercular cavity, is also believed to produce white blood cells in juvenile salmonids (Grace and Manning 1980; Chilmonczyk 1985).

Cells make up about 25 - 45% of the blood, measured as packed cell volume or hematocrit. There are two major groups of blood cells, red blood cells or erythrocytes, and white blood cells or leucocytes (Figure 3). Hematocrit varies with species, the environment, state of health, and where the blood sample is actually removed from the fish. If hematocrit is very low, this indicates a possible pathological condition, extreme hydromineral imbalance, or both. An unusually high hematocrit may be caused by swelling of cells, increased cell number, or decreased plasma fluid, and is a possible indication that the fish is in a stressed condition. A population of fish having individuals with both low and high hematocrit values such that the coefficient of variation for the sample is >15% may have a potential health problem (Goede and Barton 1990).

*1). Erythrocytes.* The concentration of red cells in salmonid blood is in the range of $1 - 2 \times 10^6$ per $mm^3$ of blood. The nucleated erythrocytes of salmonids (Figure 3) are generally about $14 \times 8$ $\mu m$ (elliptical diameter) in size (Smith 1982); by comparison, a human red blood cell is about 7.5 $\mu m$ in diameter (Sell 1987). The major function of erythrocytes is to transport $O_2$. Oxygen is carried in the haemoglobin, an iron-based pigment molecule. Haemoglobin comprises up to 70% of the dry matter of an erythrocyte (Satchell 1991). Thus, total haemoglobin content varies with blood cell count or hematocrit. The normal haemoglobin content for salmonids (e.g., rainbow trout and coho salmon) is 5.4 - 9.9 g/dl (Wedemeyer et al. 1990).

Teleost fish have multiple haemoglobins and in salmonids at least two types of haemoglobins with substantially different properties are present (Riggs 1970). Moreover, salmonids may display ontogenetic shifts in haemoglobin types with age (Giles and Vanstone 1976). The affinity of blood for $O_2$ depends on the association-disassociation capacity of the haemoglobin molecule for $O_2$. A number of factors affect the binding of $O_2$ with haemoglobin, particularly blood pH, partial pressure of $CO_2$ ($PCO_2$), and temperature. A fundamental property of haemoglobin is its ability to take up $O_2$ at the gills and then unload $O_2$ at the tissues as a result of its decreased affinity for oxygen in the presence of reduced blood pH caused by metabolic $CO_2$ (the Bohr effect, *see* Riggs 1970; Satchell 1991). Whereas the Bohr effect is a pH- or $PCO_2$-induced shift in blood oxygen *affinity*, the Root effect is a shift in blood oxygen *capacity*; the latter is now considered as an extreme Bohr shift (Moyle and Cech 1988). Unlike low-$O_2$ tolerant fish

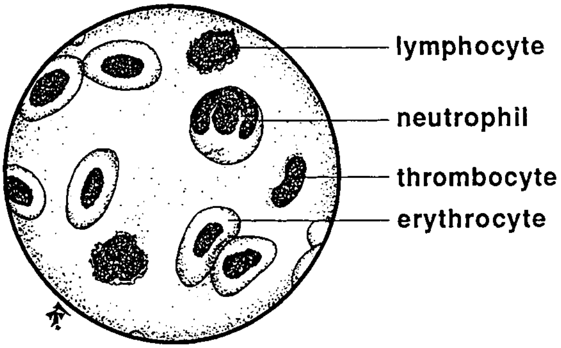

FIGURE 3. General appearance of erythrocytes, lymphocytes, thrombocytes, and neutrophils in salmonid blood as they would appear on a stained smear through a microscope (drawn from photographs in Yasutake and Wales 1983).

that have a high blood affinity at low $PO_2$, salmonid haemoglobin has a relatively low blood $O_2$ affinity. As a result, salmonids are physiologically efficient in a well oxygenated environment, which is characteristic of their typical habitats (e.g., flowing streams, oligotrophic lakes) (Moyle and Cech 1988).

*2). Leucocytes.* Several kinds of leucocytes, or white blood cells, are present in salmonid blood, ranging in number from about 8 to 21 x $10^3$ per $mm^3$ (Wedemeyer et al. 1990). The principal cell types are lymphocytes, thrombocytes, monocytes or macrophages, and granulocytes (Figure 3). Leucocytes are important in fish for conferring resistance to disease through various immune responses. Lymphocytes are the most numerous and vary in size from 4.5 to 12 $\mu$m, consisting of mostly nucleus with a narrow margin of cytoplasm (Figure 3). A major function of lymphocytes is the production of antibody. Thrombocytes are relatively small leucocytes and are involved in the blood clotting process. Monocytes, or macrophages, and granulocytes have a phagocytic action, and the latter may also have a bactericidal function. Granulocytes, in particular neutrophils, are very easy to identify in a blood smear, being relatively large with a lobular nucleus (Figure 3). Excellent colour plates of the various blood cell types in salmonids are found in Yasutake and Wales (1983). A more complete description of teleost blood cell types is provided by Houston (1990).

*3). Plasma.* The plasma is the fluid portion of the blood (serum is plasma with the fibrinogen removed by clotting). The salt fraction of plasma consists mainly of sodium ($Na^+$) and chloride ($Cl^-$), but a number of other ions, including potassium, calcium, and magnesium, are present in much smaller concentrations. Typical plasma ion levels in salmonids may range between 150 - 155 meq/l for $Na^+$ and 84 - 136 meq/l for $Cl^-$ (Wedemeyer et al. 1990). In salmonids, as in other euryhaline fish, plasma osmolality may be slightly higher in marine fish than in freshwater forms. The function of plasma is to transport dissolved minerals, digestive products, hormones and enzymes, antibodies, dissolved gases, and wastes.

*Gas (Swim) Bladder.* Salmonids are physostomous fish; that is, the gut and the gas (or swim) bladder remain connected through life by the pneumatic duct located in the esophageal region. Embryologically, the gas bladder originates as an outpocketing of the gut. Physostomous fish fill the gas bladder by forcing air gulped at the surface through the pneumatic duct. First filling of the bladder occurs at the swim-up fry stage when the yolk sac has nearly or completely disappeared. Tait (1960) found that normal filling of the gas bladder occurred from 3.5 to as long as 17 weeks after hatching in lake, brown, and rainbow trout, and that this process was temperature dependent. Bladder gas in physostomes is released as the sphincter muscles controlling the pneumatic duct relax (Moyle and Cech 1988).

The gas bladder is a hydrostatic organ that regulates and maintains neutral buoyancy. This organ has the unusual property of being able to maintain oxygen and nitrogen gas at partial pressures many times above normal atmosphere (Steen 1970). The gas bladder is characterized by the presence of a specialized capillary network, the rete mirabile, which employs a countercurrent system to unload gas from arterial blood into the bladder. The rete system is much more developed in physoclistic fish, which have lost the connection between the gut and bladder, than it is in physostomes such as salmonids (Steen 1970).

*Kidney and Spleen.* The kidney has a major role in hydromineral balance and eliminates excess water in a freshwater environment or excretes divalent ions, mainly magnesium and sulphate, in a saltwater environment (*see* Rankin et al. 1983). All salmonids possess a nephric kidney; that is, the functional unit of the kidney is the nephron — a structure designed for removing water from the blood. The kidney is particularly sensitive to infections and is useful when examining for the early detection of many pathogens.

Often the spleen is found embedded in the mesenteric fat deposits surrounding the lower stomach and intestine, usually located posterior to the pyloric caeca. In apparently healthy fish, the spleen can have either a smooth or granular appearance; the significance of the latter condition is not known but blood pooling may cause this appearance (Goede and Barton 1990).

*Liver and Gall Bladder.* The liver synthesizes complex proteins (e.g., enzymes) from digested foodstuffs, converts nutrients to different forms (e.g., proteins to carbohydrates), produces bile, and metabolizes or clears hormones (e.g., steroids). The liver is also a major storage organ for glycogen in salmonids and is very important as a site of removal of foreign compounds. Such material is removed by biotransformation (e.g., involving the mixed-function oxidase system) or formation of complexes (e.g., binding of metals with metallothionein) to forms suitable for storage or excretion (Heath 1987).

The gall bladder stores bile produced in the liver and secretes it into the gut to aid in lipid digestion. Bile consists of bile salts, cholesterol, phospholipids, glycoproteins, organic and inorganic ions, and pigments (Fänge and Grove 1979). As bile remains in the gall bladder, bilirubin, which initially imparts a yellow colour, is oxidized over time to biliverdin, which gives the bile a blue-green appearance (McCormick and Podoliak 1984). By using gall bladder fullness and bile colour as a relative index, one can estimate approximately when the fish ate last (Goede and Barton 1990).

*Nervous System.* The nervous system consists of the central nervous system, the peripheral nervous system, the autonomic nervous system, and the sensory organs. These components are common to vertebrates, so general details on their structure and function in fish are not provided here (*see* Lagler et al. 1977; Bond 1979; Bone and Marshall 1982; Moyle and Cech 1988). Major sensory systems in salmonids include the acoustico-lateralis system, the visual system, and the olfactory system.

The acoustico-lateralis system involves the lateral line and inner ear, and is important for balance and hearing. In water sound consists of two components, the near-field large particle displacement component, which is detected by tactile receptors, and the far-field pressure component, which is detected by pressure receptors. Reception by the inner ear for hearing, the semi-circular canals of the inner ear for balance, and the lateral line, all operate by mechanoreception. Neuromasts, specialized mechanoreceptors designed to detect vibration, are embedded in the semi-circular canals and along the length of the lateral line. Deformation of hair cells within the neuromast by pressure or particle displacement (i.e., for vibration detection), or by fish movement (i.e., for balance) stimulates the production of neural signals sent to the brain.

The primary component of the visual system is the eye. The retina of the teleost eye contains both rods and cones. Cone characteristics and behavioural responses to specific colours suggest that teleost fish have the capability for distinguishing colours, although evidence for colour vision *per se* appears limited (Nicol 1989). Absorption of light by retinal pigments stimulates the retinal cells to send nervous impulses to the brain via the optic nerve.

In marine adult salmonids, rhodopsin is the primary visual pigment. Juvenile fish have porphyropsin and rhodopsin, with a higher proportion of the latter. The shift in pigment systems is related to the light regime, among other environmental factors. For example, the proportion of rhodopsin increases with light. While in the ocean, anadromous salmonids have a higher proportion of rhodopsin but as the fish undergo their freshwater spawning migration, porphyropsin becomes predominant. Pigment shifts associated with migration of salmonids appear to involve the endocrine system, notably the thyroid (Munz 1971; Nicol 1989).

The sensory unit of the olfactory system is the olfactory pit, which is lined with "rosettes" of chemoreceptor-dense epithelial tissue. The chemoreceptors are extremely sensitive to amino acid-based chemicals and olfaction presumably is of major importance for salmonids in locating their natal stream (Hasler 1971). The use of the amine-based compound, morpholine, by fish culturists to "imprint" juvenile salmonids to their natal hatchery before release to encourage a high rate of adult return takes advantage of this olfactory sensitivity (Hasler 1983). Salmonids are able to detect amino acids at concentrations of $10^{-7}$ M (Rehnberg and Schreck 1986) and other compounds at even lower levels (*see* Hara 1971).

*Endocrine System*. The endocrine system is one of communication within an individual organism involving cellular-produced chemicals, or hormones. An advantage of the endocrine system as a supplement to the nervous system is that the effect of hormone action can be continuous and long-lasting, rather than short-lived like a nervous impulse. Such an effect is necessary for many physiological processes, such as maturation. Another advantage of endocrine communication is that glandular secretions can reach all cells in the body through the circulatory system, instead of only those innervated with neurons. Hormone regulation depends primarily on feedback mechanisms. Release of most hormones is controlled by feedback acting on the secretory tissue; feedback control is usually negative. Regulation of hormones and their action is also controlled by rate of uptake and conversion of the hormone at the target tissue (which depends on the specific hormone receptor activity and concentration), rate of hormone clearance from circulation, and levels of the carrier protein in the plasma.

There are two basic types of hormones. Steroid hormones and tyrosine-derived thyroid hormones are hydrophobic and are able to pass through the lipid bilayer of the membrane directly into the cell and nucleus. Peptide hormones, including more complex structures based on amino-acid derivatives, are hydrophilic and lipophobic and generally do not pass through the cell membrane but must bind to membrane receptors. In order to exert their action, most peptide hormones stimulate a second-messenger system at the cellular level, such as cyclic adenosine monophosphate (cyclic AMP), which activates a protein kinase.

While most of the endocrine glands in higher vertebrates are distinct encapsulated structures, many of the equivalent structures in salmonids and other teleost fishes are not

(Figure 4). For example, the catecholamine-secreting medulla and corticosteroid-secreting cortex portions of the adrenal gland in mammals are represented in teleosts by the chromaffin and interrenal cells, respectively, found in the head kidney tissue and near associated veins. The important glandular structures of salmonids, along with the associated hormones and their presumed major functions, are listed in Table 4. However, it is cautioned that this summary is simplifying a very complex subject, as control and action of many of these hormones may be interregulated and not independent from each other. The endocrine systems of fish and other non-mammalian vertebrates are discussed in detail in a number of good comprehensive texts (e.g., Gorbman et al. 1983; Norris 1985).

*Immune System.* The study of immunology in fish is relatively new and the availability of general references and reviews is limited (*see* Cushing 1970; Anderson 1974; van Muiswinkel and Cooper 1982; Manning and Tatner 1985; Stolen et al. 1986; Hunt and Margetts 1987). However, many good general texts and journals are available on the subject of immunology, which is a rapidly expanding field. Much of the following discussion is extracted from the text by Sell (1987).

Sell (1987) described immunity simply as the ability of an organism to defend itself against invasion and colonization by foreign organisms. Resistance to invading organisms is innate or adaptive. Innate defense mechanisms include physical barriers, such as skin, secretions, such as mucus, and inflammatory responses. Innate resistance is always present and is non-specific. Adaptive resistance, conversely, is specific to a foreign organism or antigen and is not activated in the host until stimulated by it. Functional features of the adaptive immune system are its ability to: (1) recognize many different foreign invaders specifically, (2) rapidly synthesize immune products, (3) deliver those products to the site of infection, (4) provide a diversity of mechanisms to combat infectious agents with different properties, (5) direct the defense mechanisms specifically to the invading organism rather than the host's tissue, and (6) deactivate the mechanisms when the foreign organism has been cleared (Sell 1987).

The two major forms of adaptive or specific immunity are humoral and cellular. Humoral immunity targets bacteria by the synthesis and release of specific antibodies into the bloodstream. Antibodies are specialized plasma proteins known as immunoglobulins, particularly the IgM form in teleost fishes (Cushing 1970). Immunoglobulins (Ig) that act as specific antibodies are produced by special lymphocytes called plasma mature B cells (or B-like cells) in response to a stimulus by an antigen (a molecular substance capable of inducing an immune response). The response sequence is activated through specific receptor sites for the antigen on the lymphocyte cell surface. Antigens are represented by a variety of molecules, but include chemicals associated with bacteria such as polysaccharides (e.g., bacterial capsules), proteins (e.g., microbial toxins), and lipoproteins (e.g., cell membranes). Receptor-specific antigens may be released and presented to lymphocytes by macrophages after phagocytosis of the bacterial organism. Acting with Ig in the formation of the antigen-antibody complex is another set of blood

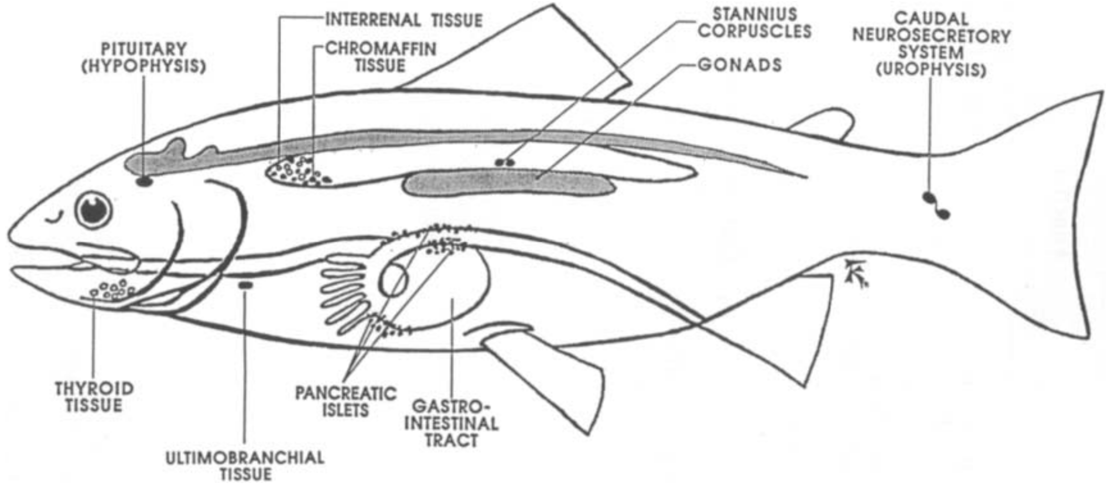

FIGURE 4. Approximate placement of the major endocrine glandular tissues in salmonid fish (adapted from Bern 1967; Anderson and Mitchum 1974).

TABLE 4

Endocrine glands or glandular tissue, their important hormones (and common abbreviations), and major hormone function (known or presumed) in salmonids.

| Glandular tissue | Hormone | Function |
|---|---|---|
| Anterior pituitary | Thyrotropin (TSH) | Stimulates thyroid tissue |
| | Prolactin (PRL) | Osmoregulatory in fresh water |
| | Growth hormone (GH, somatotropin) | Stimulates linear growth; possibly involved in salt water regulation |
| | Adrenocorticotrophic hormone (ACTH) | Stimulates corticosteroid release |
| | Gonadotropin (GTH I and II) | Stimulates steroid synthesis; involved in ovulation and spermiation |
| | $\alpha$-Melanocyte-stimulating hormone ($\alpha$-MSH) | Controls dispersion of melanophores in skin |
| | Endorphin(s) (END) | Acts as an endogenous opiate |
| | Somatolactin (SL) | Function not clearly established |
| Pineal body | Melatonin | Causes gonadal inhibition; can also affect pigmentation |
| Urophysis | Urotensins | May be ionoregulatory factor |
| Ultimo-branchial bodies | Calcitonin | Involved in calcium regulation |
| Pancreatic islets | Insulin | Lowers blood sugar |
| | Glucagon | Elevates blood sugar |
| | Somatostatin | Inhibitory factor to both of above; role not clear in fish |
| Interrenal tissue | Corticosteroids (mainly cortisol) | Major stress hormone; various actions during the stress response |
| Chromaffin tissue | Catecholamines (mainly adrenaline) | Major stress hormone; stimulates glucose release into circulation |
| Gonads | Androgens; e.g., testosterone (T), 11-ketotestosterone (11-kT)<br>Estrogens; e.g., 17ß-estradiol ($E_2$) | Sex steroids are involved in gametogenesis, vitellogenesis, maturation, and development of secondary sex characteristics |

TABLE 4 cont'd.

| Glandular tissue | Hormone | Function |
|---|---|---|
| Gonads *cont'd.* | Progestins; e.g., $17\alpha$-hydroxy-$20\beta$-dihydroprogesterone ($17\alpha20\beta$-DHP) | |
| Thyroid tissue | Thyroxine ($T_4$)<br>Triiodothyronine ($T_3$) | Growth and metabolic factor; involved in metamorphosis |
| Corpuscles of Stannius | Hypocalcin | Lowers blood calcium |
| Kidney (juxtaglomerular cells) | Renin/angiotensins | Involved in fluid and electrolyte balance |
| Gut wall | Hormone-like substances; e.g., secretin, gastrin | Involved in digestion |

proteins called complement. Complement not only acts in concert with antibodies in development of immunity and destruction of the antigen, it is considered an essential component of the immune system.

Cellular immunity is focused at viruses, fungi, and mycobacteria, and refers to the effects of the cells of the immune system directly on the foreign body. The lymphocytes involved in cellular immunity are T cells (or T-like cells). T cells are activated or sensitized after stimulus by specific antigen recognition (i.e., antigen-receptor formation as in B cells). T cells differentiate further into subpopulations depending on their functions that include, among others, assisting in antibody formation (T helper cells), killing target cells by cytotoxin formation (T cytotoxic killer cells), inducing inflammation (T delayed hypersensitivity cells), and regulating or inhibiting other immune responses (T suppressor cells). Certain antigen-activated T lymphocytes also produce specialized proteins such as interleukins, which activate or deactivate other lymphocytes, and lymphokines, which contribute to the inflammatory response (Sell 1987).

In salmonids, lymphocytes involved in the immune response are produced in the head kidney tissue, the spleen, and in juveniles, the thymus. Many fish biologists are currently interested in the immune system because of its importance in understanding the relationship between stress and disease resistance in fish (Ellis 1981; Fletcher 1981; Pickering 1987; Barton and Iwama 1991).

## ENVIRONMENTAL REQUIREMENTS

### *Temperature*

All salmonids are considered as "coldwater" fishes and their thermal optima and preferences are reflected in their temperate distribution. Because fish are poikilotherms, their metabolic rate is controlled directly by the ambient temperature. The $Q_{10}$ for teleosts is about 2.3 (Brett and Groves 1979) but may be somewhat lower in salmonids (*see* Chapter 12). ($Q_{10}$ is the metabolic rate increase factor for a 10°C increase in temperature.)

The difference between the active metabolic rate and standard metabolic rate is known as the scope for activity (Fry 1971). As temperature varies, both active and standard metabolic rates vary. The maximum scope for activity occurs, therefore, at that temperature where the difference between active and standard rates in fish is greatest. This temperature range is similar to their physiological optimum temperature. In turn, the physiological optimum for growth is often similar to the fish's preferred temperature selected behaviourally (Jobling 1981).

Temperature is perhaps the most important single determinant controlling those features that should interest aquaculturists, such as net food conversion, growth, and metabolite production. In an attempt to design guidelines to provide maximum protection for natural fish populations, criteria have been developed as single values to encompass all life stages.

These criteria are usually expressed as acute (e.g., upper acute lethal temperature - 2°C; EPA 1986) or chronic (optimum + one-third of difference between optimum and upper incipient lethal; EPA 1986) values that should not be exceeded regularly. However, optimum, preferred, and limiting temperatures depend on many other factors including duration of exposure, acclimation temperature, and life stage. Thus, it is more useful to describe lethal and optimal temperature ranges for various life stages — e.g., embryo, fry, adult — than simply a single criterion for adult fish.

A summary of temperature tolerances and preferences is presented in Table 5. In general, as upper and lower limiting temperatures are higher for one species than another, so are optimum temperatures (*see also* Chapter 7).

TABLE 5

Upper incipient or threshold lethal temperatures (°C) and physiological or behavioural optimum temperature ranges (°C) for selected salmonid species (references cited summarize data from other sources). The range in upper threshold temperature values reflects the range of acclimation temperatures used in experiments, usually from 5 to ≥20°C. Thus, fish acclimated to a mid-range value of 10-15°C can be expected to have an upper threshold temperature between the range extremes listed; the ultimate incipient lethal temperature will be at or slightly above the upper extreme value listed.

| Genus and species | Upper threshold range | Optimum range | Reference |
|---|---|---|---|
| ***Oncorhynchus*** | | | |
| pink salmon | 21.3-23.9 | | NAS (1972) |
| | | 9.3-12.8[a] | Coutant (1977) |
| | | 15.5[b] | Jobling (1981) |
| chum salmon | 21.8-23.8 | | NAS (1972) |
| | | 14.1[a] | Coutant (1977) |
| | | 13[b] | Jobling (1981) |
| coho salmon | 22.9-25.0 | | NAS (1972) |
| | | 11.4-16.6[a] | Coutant (1977) |
| | | 14.8[b] | Jobling (1981) |
| | | 9-14 | Piper et al. (1982) |
| | | 11.8-14.6 | Laufle et al. (1986) |
| sockeye salmon | 21.5-24.8 | 15[b] | NAS (1972) |
| | | 10.6-14.5[a] | Coutant (1977) |
| | | 10-15 | Piper et al. (1982) |
| chinook salmon | 21.5-25.1 | | NAS (1972) |
| | | 11.7-17.3[a] | Coutant (1977) |
| | | 15.5[b] | Jobling (1981) |
| | | 10-14 | Piper et al. (1982) |
| | | 12-18 | Raleigh et al. (1986*a*) |
| cutthroat trout | | 12-15 | Hickman & Raleigh (1982) |
| rainbow trout | 26.5 | | NAS (1972) |
| | | 13-22[a] | Coutant (1977) |
| | | 10-22 | Elliot (1981) |
| | | 16.5-17.2[b] | Jobling (1981) |
| | | 10-16 | Piper et al. (1982) |
| | | 12-18 | Raleigh et al. (1984) |
| steelhead | 23.9 | 10 | Pauley et al. (1986) |

**TABLE 5 cont'd.**

| Genus and species | Upper threshold range | Optimum range | Reference |
|---|---|---|---|
| *Salmo* | | | |
| Atlantic salmon | 22.0-23.5 | | NAS (1972) |
| | | 6-20[a] | Coutant (1977) |
| | | 12.1-15.1[b] | Jobling (1981) |
| | | 10-17 | Piper et al. (1982) |
| brown trout | 22.0-23.5 | 8-17 | NAS (1972) |
| | 22.7-26.7 (1000 min) | | Elliot (1981) |
| | 21.5-24.7 (7 day) | | Elliot (1981) |
| | | 12-17.6[a] | Coutant (1977) |
| | | 6-20 | Elliot (1981) |
| | | 10-15.5[b] | Jobling (1981) |
| | | 9-16 | Piper et al. (1982) |
| *Salvelinus* | | | |
| Arctic charr | 22-24 | | McCauley (1958) |
| | | 14[b] | Jobling (1983) |
| | | 5-16 | Elliot (1981) |
| brook trout | 23.5-25.5 | | Elliot (1981) |
| | | 13-15.4 | NAS (1972) |
| | | 8-20.3[a] | Coutant (1977) |
| | | 13-16.1[b] | Jobling (1981) |
| | | 7-13 | Piper et al. (1982) |
| lake trout | 22.7-23.5 | 16-17 | NAS (1972) |
| | | 10-15.5[a] | Coutant (1977) |
| | | 6-14 | Piper et al. (1982) |
| | | 8-15 | Marcus et al. (1984) |
| splake (hybrid) | | 12.0-13.1[a] | Coutant (1977) |
| *Hucho* | | | |
| huchen | | <15 | Holčík et al. (1988) |
| | | 16-18[b,c] | Jatteau (1991) |

[a] Final preferenda; ranges incorporate data presented from both empirical field studies and laboratory experiments. The wide ranges evident for some species probably reflect differences in stocks, fish size, acclimation temperature, and other environmental conditions. Readers should refer to source documents listed in citation for further information.

[b] Optimum for growth

[c] Fry stage (4 wk)

### *Oxygen*

Whereas temperature is a controlling factor, ambient oxygen ($O_2$) concentration is considered as a limiting factor on metabolism (Fry 1971). Salmonids generally have a higher $O_2$ demand than many other fish groups and, thus, $O_2$ criteria for protection of their life stages are high. Oxygen tolerances and requirements are similar for most salmonid species. Acute mortality of adult fish is apparent at dissolved $O_2$ concentrations between 1 and 3 mg/l (Doudoroff and Shumway 1970). However, dissolved $O_2$ concentrations higher than acutely lethal levels may affect behaviour and swimming performance. Therefore, dissolved $O_2$ criteria have been established at 4.0 mg/l as an absolute, acute (instantaneous), lower limit to prevent mortality and 6.5 mg/l as a chronic limit to protect natural salmonid populations from production impairment (Chapman 1986; CCREM 1987). For salmonid culture, the criterion of 7.8 mg/l (Davis 1975) has been suggested to ensure the maximum level of protection (DFO 1983); at 10°C, this level represents about 69% saturation.

Although $O_2$ criteria are usually expressed as concentrations, $O_2$ uptake across the gill depends on the $O_2$-tension gradient between the internal (e.g., blood) and external (e.g., water) environments. Oxygen tension, or partial pressure, depends not only on the concentration of $O_2$ in the water, but also on other physical and chemical properties of the environment, particularly water temperature, atmospheric pressure, and salinity. As the temperature increases, the ability of water to hold dissolved $O_2$ decreases. As atmospheric pressure increases, the ability of water to hold dissolved $O_2$ also increases. Thus, the solubility of $O_2$ in water decreases with both increasing temperature and altitude, and, at full saturation, the quantity of oxygen available to the fish will be less. It is therefore important to consider saturation levels as well as concentration criteria when assessing $O_2$ requirements relative to loading demands; for further discussion of this subject in the context of salmonid rearing, *see* Davis (1975), DFO (1983), or Needham (1988).

Salmonid embryos have similarly high dissolved $O_2$ requirements that change with their development. A minimum dissolved $O_2$ of 6 mg/l is recommended for eggs to the eyed stage and up to 11 mg/l (at 10°C) for eggs from the eyed stage to hatching (DFO 1983). For post-hatch stages, Davis (1975) recommended a dissolved $O_2$ level of 7.8 mg/l for the maximum level of protection.

### *Salinity*

Most salmonid fishes are anadromous or have anadromous forms, and many species have stocks that are both anadromous and entirely land-locked (e.g., cutthroat trout, Atlantic salmon, rainbow trout and steelhead, masu salmon and yamame, sockeye salmon and kokanee). Notable exceptions to intraspecific anadromy are the charrs of the genus *Salvelinus*. Well-known examples are lake trout and bull trout, although the latter species was only recently separated from its anadromous counterpart, the Dolly Varden (Cavender 1978). Lake trout stocks also occur occasionally in coastal arctic waters although they are not truly marine (McPhail and Lindsey 1970; Scott and Crossman 1973).

Regardless of their life history pattern, embryonic and larval stages of all salmonids are freshwater forms, although certain species such as chum and pink salmon migrate to the ocean early in their life history. Salmonid eggs are relatively tolerant of environmental salinity. Salinities above 24 parts per thousand (‰) may inhibit fertilization, although fertilization in some Pacific salmon has been observed at salinities as high as 30‰ (Holliday 1969).

Within tolerable limits, the ambient salinity of the environment affects metabolic rate. Rao (1968) found that when ambient salinity was increased from 0 to 7.5‰, metabolic rate was lowered in both resting and exercised rainbow trout. Those results suggest that at near-isotonic conditions when the osmotic gradient between fish and environment is minimal, the energetic cost for activity is reduced accordingly. However, this widely held view was questioned recently by Morgan and Iwama (1991) who found that metabolic rates, and also growth, of rainbow trout, steelhead, and chinook salmon were lowest in fresh water and increased with salinity. Morgan and Iwama (1991) concluded that optimal salinities for salmonid metabolism are influenced by species, life stage, and season (*see* Chapter 7).

### *Hydrogen Ion (pH)*

The term "pH" is used to describe the quantity of hydrogen ($H^+$) ions in solution and is an inverse logarithmic term (i.e., $pH = -\log [H^+]$); the more $H^+$ ions present, the more acidic the solution is and the lower the pH. Salmonids at all life stages are sensitive to changes in ambient pH beyond their tolerable range. The reduction in pH by environmental acidification such as from acid rain, for example, is responsible for the extirpation of salmonid stocks throughout North America and Europe (papers *in* Johnson 1982). Fertilization of salmonid eggs likely will not occur at pH <4.0 but abnormal embryonic development and hatching can occur at pH 5.0-5.5 (Peterson et al. 1982). Acute effects of acid exposure on salmonids are evident at pH 4.0-4.5 and are manifested by impaired ionoregulation, acid-base balance, and $O_2$ uptake (Wood and McDonald 1982). Discerning the pH that causes harmful effects on fish may be difficult, however, because of the modifying effects of water hardness, ionic strength, temperature, $CO_2$, and presence of heavy metals in the environment (Spry et al. 1981). Some salmonid species and stocks may be more resistant to low pH than others (e.g., brook trout — Robinson et al. 1976; Swarts et al. 1978) and have been considered for hatchery stocking programs in waters too low to support natural reproduction.

### *Ammonia*

Unlike many compounds that become more toxic as pH decreases, toxicity of total ammonia increases dramatically with increasing pH. This is because at higher pH values, increasingly more ammonia is dissociated from the relatively nontoxic ionic form ($NH_4^+$) to the highly toxic free form ($NH_3$). Ammonia dissociation is also temperature dependent. At pH 7.0, <1% of total ammonia is $NH_3$ whereas at pH 9.5, $NH_3$ constitutes 21% (at 0°C) to 72% (at 30°C) of total ammonia (Emerson et al. 1975). For example, if a water sample at 10°C contained 2 mg/l total ammonia, the $NH_3$ content would be <0.004 mg/l at pH 7.0, while at pH 9.5 it would be 0.74 mg/l and could cause sublethal or lethal toxic effects on salmonids. Mortality (96-h $LC_{50}$) in salmonids has been documented at $NH_3$ levels between 0.08 and 1.1 mg/l (Russo and Thurston 1991). A suggested guideline for salmonid culture is that $NH_3$ should not exceed 0.01 mg/l in the effluent (DFO 1983) and remain below 0.0125 mg/l generally for optimum health of salmonids (Piper et al. 1982). For details on $NH_3$ calculation from total ammonia at different pH and temperature values, see Emerson et al. (1975).

### *Other Environmental Factors*

A host of other ions and compounds exist that, when present in excessive amounts in the water, can be potentially harmful to salmonid fish. With most compounds, both acute and chronic toxicity to fish vary with species, life stage, acclimation conditions, and other environmental features (e.g., water hardness). However, some of the published criteria that are useful as guidelines for culturing or maintaining healthy salmonid populations are summarized in Table 6. Water quality requirements for salmonid embryos are described in Chapter 5.

## PHYSIOLOGICAL PROCESSES

### *Respiration*

In salmonid fish, virtually all respiration, or gas exchange, takes place across the gill membranes. Salmonids ventilate their gills actively and water movement is controlled by the buccal and branchial muscles. In a respiratory cycle, opening the mouth produces a small negative pressure in the buccal and branchial cavities. When the mouth closes, the resultant positive pressure in the buccal cavity forces water back into the branchial chamber and over the gills. The drop in pressure in the buccal cavity then allows water to enter when the mouth is opened again, thereby setting up a continuous pressure wave in one direction across the gills.

TABLE 6

Summary of water quality characteristics for salmonid fishes as suggested values for salmonid culture (Piper et al. 1982; DFO 1983) and/or guidelines for the protection of aquatic life in general (CCREM 1987). [SW = soft to moderately hard water (<150 mg/l); HW = hard to very hard water (>150 mg/l) (EPA 1986)] (*See also* Chapter 6).

| **Chemical feature** | **Suggested value for culture** | **Guideline for aquatic life** |
|---|---|---|
| Total gas pressure (%) | <103 | |
| pH | 6.5 - 8.0 | 6.5 - 9.0 |
| Carbon dioxide (mg/l) | <10 | |
| Hardness (mg/l $CaCO_3$) | 10 - 400 | |
| Alkalinity (mg/l $CaCO_3$) | 10 - 400 | |
| Calcium (mg/l) | 4 - 160 | |
| Chlorine (mg/l) | <0.15 | |
| Manganese (mg/l) | <0.01 | |
| Nitrite (mg/l) | | 0.06 |
| Phosphorous (mg/l) | 0.01 - 3.0 | |
| Hydrogen sulphide (μg/l) | <1.0 | |
| Iron (mg/l) | <0.15 | 0.30 |
| Aluminum (mg/l) | | 0.005 (pH <6.5)<br>0.1 (pH >6.5) |
| Copper (μg/l) | | 2 (SW)<br>3 - 4 (HW) |
| Cadmium (μg/l) | | 0.2 - 0.8 (SW)<br>1.3 - 1.8 (HW) |
| Lead (μg/l) | | 1 - 2 (SW)<br>4 - 7 (HW) |
| Nickel (μg/l) | | 25 - 64 (SW)<br>110 - 150 (HW) |
| Mercury (μg/l) | | 0.1 |
| Zinc (mg/l) | <0.05 | 0.03 |
| Suspended solids (mg/l) | <3 (incubation)<br><25 (rearing) | <10% above background |

Oxygen transfer across the gill is directly proportional to its surface area but inversely proportional to its thickness (Hughes 1984). Total gill area available for respiration varies among fishes and is usually related to their degree of activity. Active fishes generally have greater gill surface area per unit body weight than sedentary species. As calculated by regression, an equation for defining the gill surface area of a representative salmonid, the rainbow trout, is: $Y = 314.8W^{0.932}$, where $Y$ = total gill area ($mm^2$) and $W$ = weight (g) (Hughes 1984). Only a portion of the gill lamellae are perfused with blood under normal conditions, but during conditions of increased activity or hypoxia, additional lamellae are "recruited" as required. Lamellar recruitment is apparently an adrenergic response (Booth 1979).

Water contacts the lamellar surface for a brief period of time (Randall and Daxboeck 1984) during which gas exchange takes place by simple diffusion across the epithelial membrane, the water-blood barrier. The exchange of gases across the lamellar epithelium is facilitated by a countercurrent arrangement of blood and water flow; that is, blood flow in the lamellae and water flow through the interlamellar spaces are in opposite directions. Models for countercurrent flow in fish gills were described in detail by Piiper and Scheid (1984).

Passive diffusion of $O_2$ across the lamellar epithelium is related to the partial pressure of $O_2$ ($PO_2$) in the blood relative to that in the water. Diffusion is assisted by the oxygen-binding capacity of haemoglobin. Haemoglobin can hold 10-20 times more $O_2$ in bound form than water can. Because of haemoglobin's reversible $O_2$-binding characteristics, large quantities of $O_2$ can be bound at the gills and released at metabolizing tissue sites with relatively small changes in blood $PO_2$.

Ventilatory volume (i.e., amount of water passing over the gills) and cardiac output (which determines blood flow through the gills) affect the rate of gas exchange. Ventilatory volume can be altered by changing both the frequency of ventilatory activity and the amplitude of ventilation. Cardiac output can be altered by changing both the heart rate and stroke volume.

Environmental factors of temperature and dissolved $O_2$ and $CO_2$ levels also affect the rate of gas exchange across the gill surface. As discussed earlier, both temperature and $PCO_2$ affect the binding affinity and capacity of haemoglobin for $O_2$, and thus the fish's ability to take up $O_2$. Metabolic rate is usually measured indirectly and expressed as $O_2$ consumption rate (e.g., mg $O_2 \cdot kg\ fish^{-1} \cdot h^{-1}$) (Cech 1990), and it is influenced by temperature (e.g., $Q_{10}$ of fish $\approx 2.3$, as discussed earlier), life stage of the fish, nutritional status, and stress.

Nutritional status affects the rate of $O_2$ consumption because of the additional metabolic demand placed on the fish in order to digest food. Metabolic rate is much higher just after consumption of food than when fish are in a post-digestive state. This period of post-feeding elevated metabolic rate may last as long as 48 h, but in salmonids in general, it is about 24 h (Beamish 1978; Brett and Groves 1979).

Although other factors affect metabolic rate, such as dissolved $O_2$ levels, salinity, and diurnal periodicity (Brett and Groves 1979), one factor that should concern culturists is stress from physical disturbance caused by practices such as handling and transport

(Barton and Iwama 1991; *see* Chapter 12). For example, Barton and Schreck (1987) showed that even brief handling was sufficient to increase the metabolic rate of steelhead by about 25%. Fish culturists need to be aware of factors that increase fishes' metabolic demands, and thus $O_2$ uptake, in order to be able to accurately calculate loading rates, particularly when the decision is based solely on ambient $O_2$ levels in the water.

### *Ionic and Osmotic Regulation*

*Marine Environment.* In the marine environment, salinity of the water is considerably higher than that of internal body fluids. Thus, water tends to be drawn out and salts tend to diffuse inwards. Salmonids and other teleosts regain water lost to the environment by drinking seawater. Drinking satisfies the fluid requirement but fish then have to eliminate the excess salt. Salt is removed by active ion transport across the lamellar and filamental epithelia via specialized mitochondria-rich chloride cells (Conte 1969; Zadunaisky 1984).

Sodium ions from the blood stream are actively pumped against the concentration gradient from the cell into the serosal space around the cell. Sodium ions move from the area of high concentration in the serosal side of the cell to the external environment down a concentration gradient via sodium channels. Energy to drive the sodium pump comes from the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), a reaction catalyzed by the enzyme sodium/potassium adenosine triphosphatase ($Na^+/K^+$-ATPase). In order to maintain neutrality, $Cl^-$ diffuses directly from the chloride cell into the apical space and external environment. The activity of the chloride cell is probably under hormonal control.

In addition to $Na^+$ and $Cl^-$, fish also ingest a variety of other ions in lesser amounts when they drink salt water. Divalent salt ions, for example, sulphate ($SO_4^{2-}$) and magnesium ($Mg^{2+}$), are eliminated via the distal tubule in the kidney and excreted in highly concentrated urine.

*Freshwater Environment.* In fresh water, salmonids are hypertonic with their environment; that is, body salinity is higher than that of the surrounding medium. The fish tend to lose salts and take up water through the gills, and therefore must be able to eliminate excess water. Freshwater salmonids get rid of excess water by producing large quantities of dilute urine.

Teleosts have a nephric kidney, which contains functional nephrons. The nephron functions to remove water using a "high pressure" system. By restricting the flow of blood within the capillary network of Bowman's capsule, the functional unit of the nephron, water is forced from the blood and collected in the capsule, which drains into the renal tubule. In the proximal part of the renal tubule, sodium ($Na^+$) and glucose are conserved by reabsorption via active transport. In the distal tubule, excess $H^+$ and $NH_4^+$ may be also eliminated.

In order to maintain an internal salinity greater than the environment, freshwater salmonids also pump $Na^+$ and $Cl^-$ actively from the external environment inward against the concentration gradient. This active transport system appears to be linked to excretion of excess ammonium ($NH_4^+$), $H^+$, and bicarbonate ($HCO_3^-$) ions.

### *The Parr-Smolt Transformation (Smoltification)*

Salmonids that are truly anadromous undergo a physiological metamorphosis that enables them to osmoregulate in a saltwater environment after an early life history in fresh water. This change, known as the parr-smolt transformation, or smoltification, is how the fish become functional smolts capable of a marine existence (reviews by Folmar and Dickhoff 1980; Langdon 1985; McCormick and Saunders 1987; Hoar 1988; *see* Chapter 8). Some salmonids are capable of seawater entry shortly after emergence (e.g., chum and pink salmon) whereas others maintain their freshwater existence for one or more years before becoming smolts (e.g., coho, chinook, sockeye, masu, and Atlantic salmon, and steelhead) (*see* Chapter 3).

A major change that occurs during smoltification is a dramatic increase in the number and activity of chloride cells in the gills (Folmar and Dickhoff 1980; Hoar 1988). An increase in $Na^+/K^+$-ATPase activity indicates chloride cell activity and monitoring this activity is one of the useful smolt indicators. Accompanying these anatomical and physiological changes during smoltification, and also useful as smolt indicators, are increases in plasma levels of certain hormones, notably thyroxine (Folmar and Dickhoff 1980; Dickhoff and Sullivan 1987; Young et al. 1989) and cortisol (Specker and Schreck 1982; Barton et al. 1985; Young et al. 1989). Behavioural changes, such as saltwater preference and migratory behaviour (Folmar and Dickhoff 1980), often become evident as the juvenile fish become smolts.

Knowing when smoltification occurs is useful in salmonid culture, particularly when preparing to stock juveniles into marine grow-out facilities. Moreover, the ability to determine this critical period is important in salmonid fisheries enhancement programs as it is suspected that anadromous and potamodromous (migratory in fresh water) salmonids imprint to their natal streams during smoltification (Hasler 1983).

In addition to monitoring $Na^+/K^+$-ATPase activity and hormone titres to determine smoltification, other simpler techniques are available. As salmonids undergo smoltification, their length-weight ratio, as determined by condition factor ($K = [g \times 10^5]/mm^3$), becomes significantly reduced (Vanstone and Markert 1968; Fessler and Wagner 1969). A more complex approach to measuring changes in body conformation is body truss analysis (Winans and Nishioka 1987). Changes in general body shape may be monitored by comparing measurements of morphometric distances between "landmark" points (e.g., anterior and posterior fin insertion points) in a truss network using principal component analysis.

Smoltification can also be determined by monitoring body silvering. As salmonids undergo the smoltification process, they become silvery in appearance. Silvering is caused by the deposition of hypoxanthine and guanine crystals, particularly the latter, in two skin layers. This process accelerates during the period of smoltification (Hoar 1988).

A performance-monitoring method to evaluate functional smolts is the saltwater-challenge test (Clarke 1982; Blackburn and Clarke 1987). The saltwater-challenge test is used to determine if smolts are capable of regulating their blood $Na^+$. Fish are placed into a known concentration of salt (NaCl) water and blood samples are taken from fish 24 h later for $Na^+$ analysis. If the fish are capable of regulating blood $Na^+$ levels to <170 meq/l, they are considered as functional smolts (*see* Chapter 8).

The period of smoltification is a stressful one for juvenile salmonids. During this period, salmonids are more sensitive to handling stress than parr, or pre-smolts, (Barton et al. 1985) and their immune capacity is significantly reduced (Maule et al. 1987). Because of this they are likely to be more susceptible to disease than other life stages are. Salmonids are usually transferred from fresh water to seawater during this critical period, so culturists should take precautions to ensure that stressors encountered during this time (e.g., handling, grading, transport) are minimal.

### *Acid-Base Balance*

The ultimate goal of acid-base regulation is to preserve the correct net charge on proteins, such as enzymes. This is critical to their biological function (Spry et al. 1981) and maintenance of proper acid-base balance is important for all metabolic processes.

In fish, acid-base balance is regulated primarily by the bicarbonate-carbonic acid buffer system. The relationship among the components of this buffer system is described the Henderson-Hasselbalch equation, as follows:

$$\mathrm{pH} = \mathrm{pK'} + \log ([\mathrm{HCO_3}^-]/[\alpha\mathrm{CO_2} \times \mathrm{PCO_2}]) \qquad (2)$$

where: pH = blood pH ($H^+$ ion concentration), pK' = apparent pK, $\alpha CO_2$ = solubility coefficient of $CO_2$, $[HCO_3^-]$ = bicarbonate ion concentration, and $PCO_2$ = partial pressure of dissolved $CO_2$. Although pK' and $\alpha CO_2$ vary with temperature, their ranges are small. Thus, acid-base balance in the blood is effectively maintained by regulating either the bicarbonate ion or free $CO_2$ in the blood. However, controlling free $CO_2$ through ventilation is relatively ineffective. Because $CO_2$ is much more soluble than $O_2$

in water, $CO_2$ is "washed out" at the gills during normal ventilation. Therefore, in order to keep normal $O_2$ levels in the blood, $PCO_2$ remains relatively low and varies little.

Most acid-base regulation is accomplished by altering $HCO_3^-$ in the blood through transepithelial ion transfer across the gill. But because free $CO_2$ in the blood is low, $HCO_3^-$ is also kept low (according to terms of the Henderson-Hasselbalch equation). In short, salmonid blood is poorly buffered and is sensitive to metabolic and environmental changes in pH, such as that caused by acidic precipitation, exercise, or environmental $CO_2$.

### *Digestion and Excretion*

The digestive process begins once food has entered the mouth, where food is broken down mechanically by the teeth. Mucus secreted in the esophagus aids swallowing and the food is broken down chemically in the stomach. Here, complex proteins are split into component polypeptides mainly through the action of pepsin, a digestive enzyme secreted by cells in the gastric mucosa, the innermost lining of the gut wall. Specialized glandular tissue also secretes hydrochloric acid to keep stomach pH low (pH 2-4) in order to allow pepsin to work effectively. Gastric secretions are initiated mainly by stimulation from stomach distention and are under vagus control (Weatherley and Gill 1987).

From the stomach proper, partially digested food passes into the alkaline environment of the intestine. The numerous pyloric caeca (Table 3) arising from the ascending intestine immediately posterior to the salmonid stomach greatly increase the area of the digestive surface. In an environment of pH 7-8, trypsin and chymotrypsin, secreted by the pancreatic tissue, break down polypeptides into short polypeptide chains (Weatherley and Gill 1987). Intestinal peptidases and pancreatic carboxypeptidases break down these polypeptides eventually into the component amino acids. Amino acids are passed into the mucosal cells by active transport and subsequently diffuse into the circulatory system.

Lipids are digested initially by bile salt secretions, produced in the liver and stored in the gall bladder, which break up fat globules into smaller particles to form micelles with the salts. Lipases, secreted by the pancreatic tissue, hydrolyze neutral fat (triglycerides) into mono- and diglycerides, glycerol, and free fatty acids (Weatherley and Gill 1987). When a micelle contacts the mucosal cells at the brush border in the intestinal wall, free fatty acids and glycerides diffuse through the lipophilic membrane directly into the cell.

Carbohydrases are also present in the gut for breaking down carbohydrates into their component starches and simple sugars. For example, amylase, secreted from the pancreatic tissue, breaks down starch.

Waste products of digested food materials are ammonia (from protein breakdown), and water and $CO_2$ (from lipid and carbohydrate breakdown). Water is excreted or reabsorbed, and ammonia and $CO_2$ are eliminated at the gills. The production of ammonia is a particularly effective way to eliminate nitrogenous waste because: (1) free ammonia diffuses easily across the gill membrane, (2) ionized ammonia can be exchanged for $Na^+$ and thus removed by active transport, and (3) less energy is required to convert nitrogenous compounds to ammonia than to a more complex form such as urea. Undigested food material is excreted as feces.

### *Natural Diets and Nutrition*

Salmonids can be classed as euryphagous carnivores; that is, they feed on a wide variety of animal matter. Thus, salmonids may be planktivores, insectivores, or piscivores, or a combination of these depending on species, stock, and size. Salmonids are also considered as opportunistic feeders and food consumption depends on: (1) the caloric value of the food, (2) their body size, and (3) environmental temperature. Salmonid fry ($<3$ g) can consume up to 20% of their body weight (dry) per day whereas large adults ($>1$ kg) may only consume a maximum of $<1\%$ of their body weight per day (Brett 1979). Table 7 shows examples of major diet preferences for different salmonid species (*see also* Chapter 3).

Salmonids often undergo major changes in feeding behaviour as they grow and develop. Such shifts are referred to as feeding stanzas and may be reflected in a noticeable change in growth rate as the fish switches to a more energetically efficient food source, for example, from insects to fish.

In quantitative terms, protein is the most important dietary requirement of salmonids. The optimum protein to calorie ratio is almost double that of mammals (Brett and Groves 1979). Lipids are of lesser importance in salmonids' natural diet whereas carbohydrate requirements are low. Salmonids are not capable of digesting large quantities of carbohydrate in their diet (*see* Chapter 10).

Body composition of salmonid fish will reflect their diet and may also vary depending on their feeding activity, muscular activity, and environment (Love 1970). Salmonids from a wild environment may be significantly different, for example, in body composition, compared with those from a hatchery environment (Table 8).

TABLE 7

Preferred natural diets of selected salmonid fishes, including juvenile and adult, and freshwater and marine forms (references given are synopses that cite original papers).

| Species | Preferred food items | References |
|---|---|---|
| Pink salmon | chironomids, ephemeropterans, plecopterans, ostracods, amphipods, copepods, invertebrate eggs, euphausiids, fish | Raleigh & Nelson (1985); Bonar et al. (1989) |
| Chum salmon | chironomids, simuliids, plecopterans, ephemeropterans, trichopterans, amphipods, copepods, fish | Hale et al. (1985) |
| Coho salmon | dipterans, ephemeropterans, plecopterans, decapods, amphipods, euphausiids, crab larvae, fish, (e.g., smelt, clupeids, juvenile salmonids) | McMahon (1983); Laufle et al. (1986) |
| Masu salmon | copepods, amphipods, mysids, schizopods, euphausiids, squid, insects, salmonids, herring, capelin, mackerel, other fishes | Tanaka (1965); Kato (1991) |
| Sockeye salmon | cladocerans, copepods, euphausiids, crab larvae, salmon, mysids, amphipods, Pacific sand lance | Pauley et al. (1989*b*) |
| Chinook salmon | cladocerans, copepods, gammarids, insects, salmon, euphausiids, larger crustaceans, larval crabs, fish (e.g., clupeids) | Allen & Hassler (1986) |
| Golden trout | chironomids, trichopterans, other insects, cladocerans, copepods | Sigler & Sigler (1987) |
| Cutthroat trout | aquatic and terrestrial insects, zooplankton, trout, fish (e.g., salmonids, sticklebacks) | Hickman & Raleigh (1986); Pauley et al. (1989*a*) |
| Sea-run cutthroat trout | gammarids, sphaeromids, isopods, shrimp cutthroat, immature crabs, fish (e.g., salmonids, clupeids, trout, sculpins) | Pauley et al. (1989*a*) |
| Rainbow trout | aquatic and terrestrial insects, zooplankton, trout, benthic fauna, fish | Raleigh et al. (1984) |

TABLE 7 cont'd.

| Species | Preferred food items | References |
|---|---|---|
| Steelhead | ephemeropterans, trichopterans, dipterans terrestrial insects, amphipods, squid, fish (e.g., juvenile salmonids, greenling) | Barnhart (1986);<br>Pauley et al. (1986) |
| Atlantic salmon | ephemeropterans, trichopterans, chironomids, salmon, simuliids, euphausiids, gammarids, decapods, clupeids, smelt, capelin, small mackerel | Wheeler (1969);<br>Scott & Scott (1988) |
| Brown trout | ephemeropterans, trichopterans, plecopterans, terrestrial insects, zooplankton, amphipods, other crustaceans, fish (e.g., clupeids) | Raleigh et al. (1986*b*) |
| Arctic charr | insects (chironomids), mysids, amphipods, fishes | Johnson (1980) |
| Bull trout | trichopterans, ephemeropterans, dipterans, other benthos, mysids, fish (e.g., salmonids, whitefish, sculpins, percids) | Goetz (1989) |
| Brook trout | benthic and drifting aquatic invertebrates, terrestrial insects, fish | Raleigh (1982) |
| Lake trout | dipterans, zooplankton (e.g., mysids), crayfish, fish (e.g., sculpins) | Marcus et al. (1984) |
| Huchen | fish (e.g., nase, grayling, barbel, gudgeon, cyprinids, pike, sculpins, eels), insects, amphibians | Muus (1971);<br>Holčík et al. (1988) |

## ENERGETICS AND GROWTH

### *Bioenergetics*

In simplistic terms, bioenergetics is the fate of digested food. The energy budget of fish is described by the following formula, or in some equivalent form:

$$I = E + M + G \qquad (3)$$

TABLE 8

Body composition, as percentage of total dry matter, of salmonid fishes from natural or hatchery environments.

| Substance | Percent of dry matter | |
|---|---|---|
| | Wild fish | Hatchery fish |
| A. Brook trout (calculated from Phillips et al. 1956 *in* Love 1970): | | |
| Protein | 76.0 | 64.6 |
| Lipid | 12.2 | 25.9 |
| Ash | 11.8 | 9.4 |
| B. Average of 10 salmonid species (from Wood et al. 1957 *in* Love 1970): | | |
| Protein | 75.8 | 68.1 |
| Lipid | 13.4 | 22.5 |
| Ash | 12.3 | 9.7 |

where I = energy ingested or ration, E = energy lost through excretion, M = energy for metabolism, and G = energy used for growth (Brett and Groves 1979). Excretion includes fecal ($E_F$) and non-fecal ($E_N$) wastes. Metabolism includes standard metabolism ($M_S$), energy expended for activity ($M_A$), and that required for internal processing of food materials (heat increment, $M_H$). Growth includes both somatic (production of body tissue) and gametic (development of sexual products) growth (*see also* Figure 5 and Chapter 7).

Energy for standard metabolism is that required by the fish to simply exist (i.e., zero level of activity). As standard metabolism is difficult to measure in practice, this value is usually estimated from $O_2$ consumption measurements made of fish at various levels of activity, with the resultant plot extrapolated to the zero activity level. In actuality, most direct metabolic rate measurements made on "inactive" fish are more appropriately called routine metabolism, which includes spontaneous activity (Cech 1990). Values for weight-specific standard metabolism determined for salmonids fed normal rations range from about 80 to 120 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ (Brett and Groves 1979). Routine metabolic rates are about 1.5 to 2 times higher than standard rates. The rate of metabolism increases with increasing size according to the relationship:

$$Y = a X^b \qquad (4)$$

where Y = metabolic rate (*not* weight-specific, e.g., mg $O_2 \cdot h^{-1}$), X = body weight, and a and b are constants. The index b is about 0.86 in teleosts in general (Brett and Groves 1979) but ranged from 0.79 to 1.15 in a limited number of salmonids (Glass 1969). The weight-specific metabolic rate (e.g., mg $O_2 \cdot kg^{-1} \cdot h^{-1}$), however, decreases as fish grow larger; that is, small fish consume more $O_2$ *per unit weight* than large fish (Cech 1990).

Energy for activity is that portion of the energy budget required to satisfy energetic needs for all activities beyond standard metabolism. Such activities include prey capture, routine swimming, feeding, digestion, and excretion, among others. Heat increment is the energy component associated with food digestion. An unavoidable energy cost is associated with the biotransformation of food, which includes deamination of proteins and "processing" of lipids and carbohydrates.

When other energy requirements have been satisfied, the amount of energy remaining in the energy budget is used for somatic or gametic growth (Figure 5). Brett and Groves (1979) derived a formula for carnivorous fish, such as salmonids, that indicated energy intake is partitioned as 44 ±7% for metabolism and 29 ±6% for growth, while 27 ±3% is excreted as fecal or nitrogenous waste.

### *Growth*

Growth may be most easily defined as the elaboration of tissue. Brett (1979) described growth as the net outcome of a series of behavioural and physiological processes that terminate with the deposition of animal substance. Although fish culturists are concerned primarily with body, or somatic growth, the energy available for growth is also directed to the development of sexual products, or gametic growth. In the present context, however, growth shall refer specifically to somatic growth.

Determining the efficiency of growth relative to the amount of food consumed is relatively straightforward and is the amount of growth expressed as weight gain divided by the weight of the food consumed, or gross conversion efficiency. The net conversion efficiency is somewhat more complicated to determine, however, as it is the amount of food consumed minus the maintenance ration. Thus, assessing net conversion efficiency requires knowledge of the amount of food needed to satisfy all metabolic requirements but still result in zero net growth.

Growth can be measured in a number of ways, the simplest being to compare changes in length or weight over time. Ricker (1979) summarized various methods for expressing such changes numerically. Absolute growth is the increase in size (w = weight, for example), or:

$$w_2 - w_1 \quad (\text{e.g., g}) \qquad (5)$$

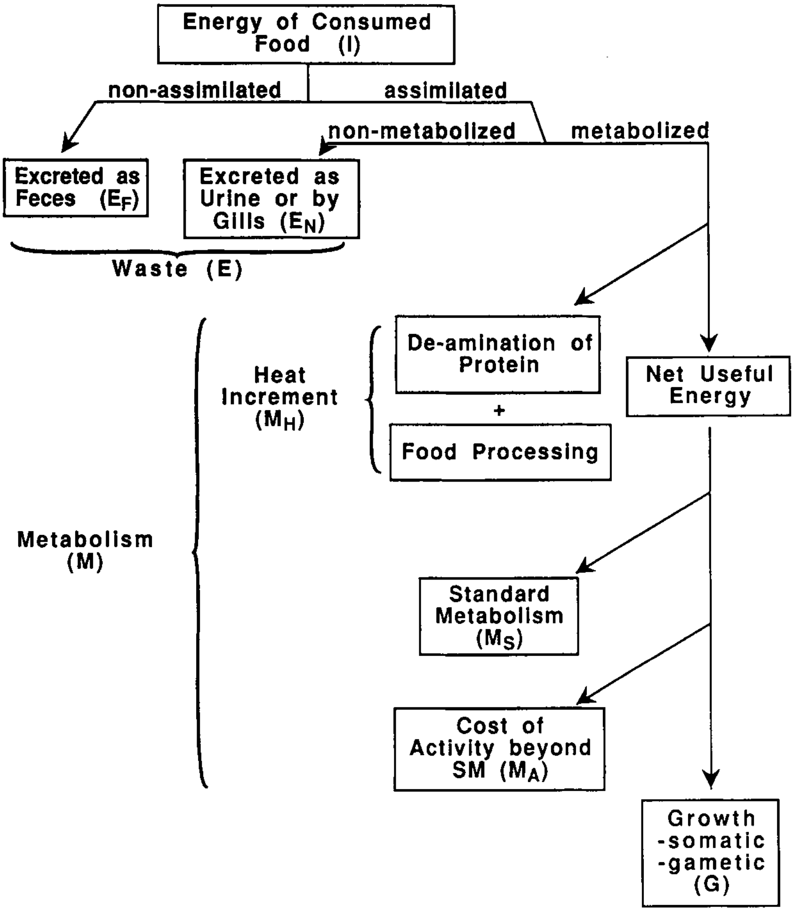

FIGURE 5. Pathway of the fate of dietary energy in fish. For typical quantitative values for each bioenergetic component in salmonids, see Brett and Groves (1979).

The absolute growth rate is the increase in size within a specified unit of time, or:

$$\frac{w_2 - w_1}{t_2 - t_1} \quad \text{(e.g., g/day or g·day}^{-1}\text{)} \qquad (6)$$

Relative growth is the increase in size relative to its initial size, whereas the relative growth rate is the relative growth within a unit period of time, or:

$$\frac{w_2 - w_1}{w_1(t_2 - t_1)} \quad \text{(e.g., mg·g}^{-1}\text{·day}^{-1}) \qquad (7)$$

Figure 6 shows the patterns of these expressions. While juvenile fish before maturation exhibit the greatest absolute weight gain and absolute growth in the exponential phase of the growth curve, the highest relative growth rate will be demonstrated by the embryonic stage because of the initial small size of the embryo (Figure 6).

In fisheries management, a useful term is the instantaneous, or specific, growth rate (G) at a particular instant in time rather than over a specified interval, and is expressed:

$$w = ae^{Gt} \qquad (8)$$

and is calculated from the formula:

$$G = \frac{\ln(w_2) - \ln(w_1)}{t_2 - t_1} \qquad (9)$$

This term G, however, is not used extensively in fish culture but is used by fishery biologists to describe and compare characteristics of natural fish populations.

Two terms, condition factor and the length-weight relationship, are used more often in fish culture and fisheries management, respectively, than the instantaneous growth rate (Carlander 1969; Piper et al. 1982; Goede and Barton 1990). While not growth terms *per se*, both provide an index of the weight to length ratio and can be used to estimate weight from length data. Condition factor is calculated as follows (C = 36.13K; Piper et al. 1982):

$$C \text{ (English units)} = \frac{w\text{(lb)}}{l^3 \text{ (in)}} \qquad (10)$$

$$K \text{ (metric units)} = \frac{w \text{ (g)}}{l^3 \text{ (mm)}} \qquad (11)$$

For convenience, both terms are often multiplied by a power of 10 in order to express the index at unity (e.g., g × $10^5/\text{mm}^3$). The length-weight relationship is determined from the expression:

$$w = al^b \qquad (12)$$

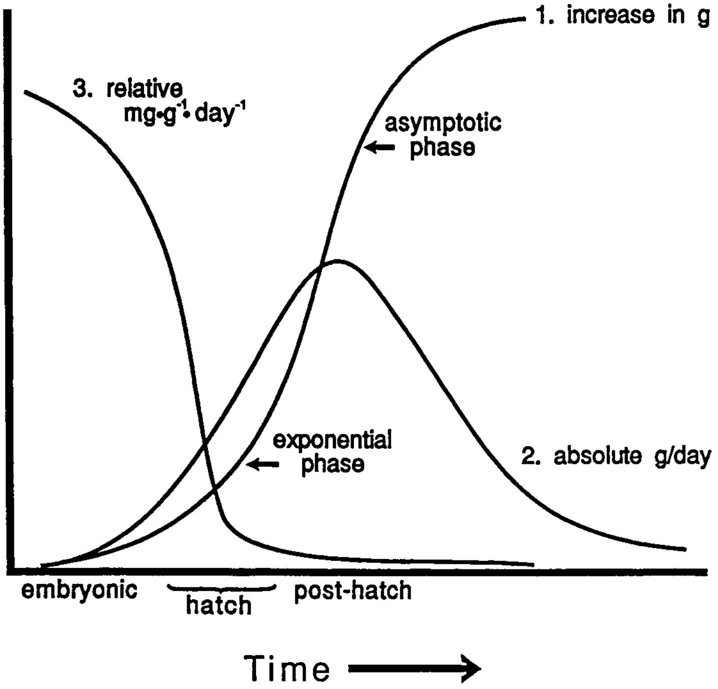

FIGURE 6. Patterns of growth described by the formulae for: 1. absolute growth (equation 5), 2. absolute growth rate (equation 6), and 3. relative growth rate (equation 7).

or:

$$\log(w) = \log(a) + b\log(l) \tag{13}$$

where b is the slope of the fitted line when the logarithm of the weight is plotted against the logarithm of the length (Carlander 1969; Ricker 1979).

When growth is isometric, i.e., the shape and specific gravity of the fish remain the same throughout its life, the slope or "b" value will be 3.0 since the weight of an object varies as the cube of its length. This does not necessarily occur in nature, however, as fish often change shape (e.g., become plumper) as they grow, or grow allometrically (Everhart and Youngs 1981). Moreover, the length-weight relationship will likely change as fish pass through different growth stanzas (such as caused by shift in food habits, change in thermal regime, metamorphosis, etc.). Carlander (1969) demonstrated diagramatically the difference in body shape and condition factor that would occur as fish grow when the slope of the length-weight relationship varies from 3.0 (Figure 7). The calculated b value for most natural salmonid populations is between 2.5 and 3.5, and usually within 2.8 to 3.2 (Carlander 1969).

Length is used most frequently to generate growth curves of natural populations. This is partly because length data are relatively easy to collect from natural populations either by monitoring commercial and recreational fisheries or index netting. By plotting length-at-age data, the pattern of absolute growth can be established for different species or stocks [Figure 8(a)]. Typical growth patterns of any species are misleading, however, as there may be as much variation in growth within a species as among species because of environmental conditions [Figure 8(b)].

In the absence of continuous year-to-year data sets for length, growth patterns may still be determined for a population from a sample of fish by back-calculation from bony structures such as scales or otoliths, assuming a direct proportion between the growth of the bony structure and somatic growth exists. In this method, fish lengths from previous years are estimated by proportion from the amount of growth determined for a particular age annulus, using the formula:

$$L_n = a + \frac{(L_t - a)(S_n)}{S_t} \qquad (14)$$

where $L_n$ is the fish length at n years, $L_t$ is the fish length at the time of capture, $S_n$ is the scale radius distance from the focus to the nth annulus, $S_t$ is the total scale radius from the focus to the edge, and a is a constant applied to correct for the length attained before scales are laid down (Everhart and Youngs 1981; Moyle and Cech 1988). The correction factor (a) is determined by plotting body length against scale measurement and extrapolating the resultant regression line to the body length (y) axis.

Growth of fish can also be determined biochemically. One method is by measuring the RNA-DNA ratio. This approach is based on the fact that DNA content of cells is constant whereas RNA content varies with the activity of protein synthesis and, thus, tissue elaboration (Bulow 1987; Busacker et al. 1990). The uptake of radioactively labeled amino acids, such as [$^{14}$C]glycine by scales, is also used as a measure of instantaneous growth rate of fish over a relatively short period of time (Adelman 1987; Busacker et al. 1990).

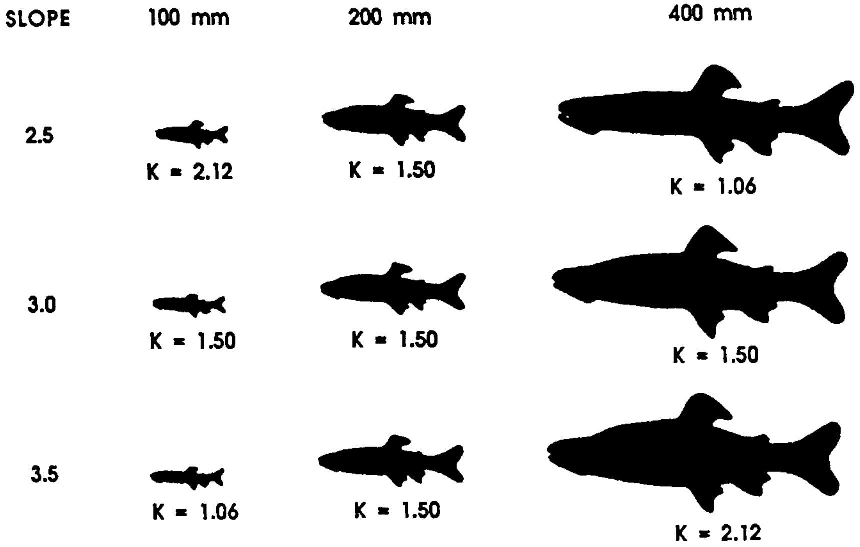

FIGURE 7. Differences in body shape of theoretical salmonids of 100, 200, and 400 mm when the slope (b) of the length-weight (log) regression is 2.5, 3.0, and 3.5 (modified from Carlander 1969). Theoretical condition factors (K) are indicated.

In addition to the genetic determinants of species and stock, numerous environmental factors affect growth. Given adequate food, the most important environmental growth factor is temperature. There is an optimum temperature range for growth in salmonids where the greatest net conversion efficiency and "scope for growth" (Warren and Davis 1967; Brett 1979) occurs. Optimum temperatures for growth will be in the range of those physiological and behavioural optima determined experimentally or empirically for individual species (e.g., Table 5). When temperatures are low, metabolic rates are greatly reduced, digestion rate is depressed, and gross conversion efficiency is low. At temperatures above the optimum range, gross conversion declines, appetite is reduced, and energetic demands for other aspects of metabolism exceed that available for growth (Brett 1979).

Whereas temperature is considered as a controlling factor on activity, including growth, ambient $O_2$ concentration is a limiting factor (Fry 1971). At or near saturation, dissolved $O_2$ levels will not likely affect growth, but at reduced $O_2$ tensions, growth rates in salmonids may be limited (Table 9). Ambient salinity also affects the growth of salmon-

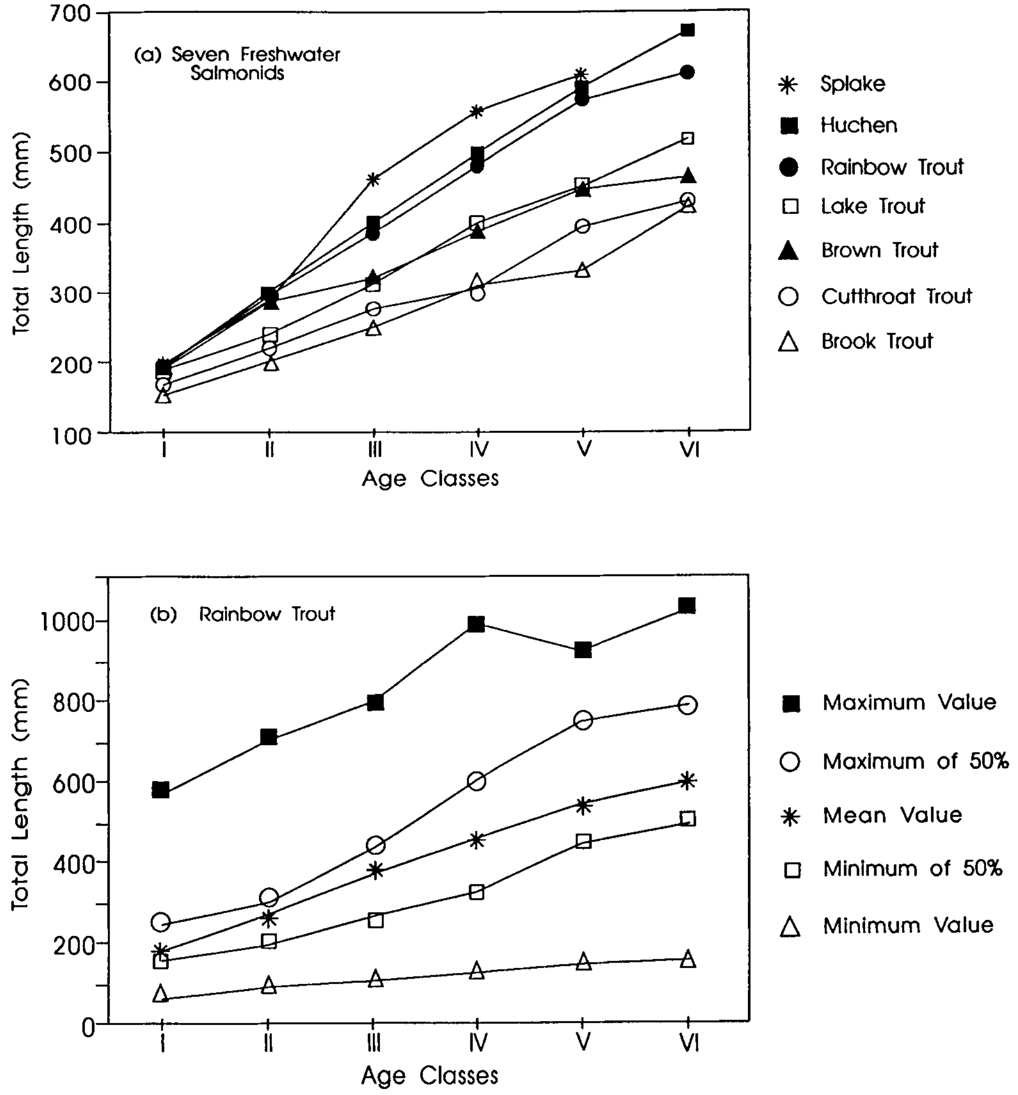

FIGURE 8. (a) Combined unweighted mean length-at-age calculated from mean values provided in Carlander (1969) for global populations of six freshwater salmonids, and average mean length-at-age values for huchen from Holčík et al. (1988). (b) Mean, minimum, and maximum values, and minimum and maximum values for 50% of the data around the mean, of length-at-age given in Carlander (1969) for global populations of rainbow trout.

TABLE 9

Percent reduction in growth rate of salmonids at various dissolved oxygen concentrations expressed as the median value from n tests (n in parentheses) for each species (from Chapman 1986).

| **Dissolved oxygen (mg/l)** | **Chinook salmon (6)** | **Coho salmon (12)** | **Sockeye salmon (1)** | **Rainbow trout (2)** | **Brown trout (1)** | **Lake trout (2)** |
|---|---|---|---|---|---|---|
| 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 1 | 0 | 0 |
| 7 | 1 | 1 | 2 | 5 | 1 | 2 |
| 6 | 7 | 4 | 6 | 9 | 6 | 7 |
| 5 | 16 | 11 | 12 | 17 | 13 | 16 |
| 4 | 29 | 21 | 22 | 25 | 23 | 29 |
| 3 | 47 | 37 | 33 | 37 | 36 | 47 |
| Median Temp. (°C) | 15 | 18 | 15 | 12 | 12 | 12 |

ids. As one would expect, the growth of parr is best in fresh water (0‰ salt) whereas that of functional smolts and anadromous adults is best at or near the concentration of seawater (25-35‰ salt) (Figure 9).

Other biotic and abiotic factors that affect growth in salmonids are age, size, season, migratory and spawning activity, and interspecific and intraspecific interactions. The effects of these factors, however, are based on the presumption that rations are adequate. If food is limited in availability, growth will be reduced and the growth response to other environmental factors may be altered. Food intake is also limited regardless of its availability and is self-regulated (Peter 1979). In natural populations, growth rates and size of energy reserve depots are correlated ultimately with the availability and nutritional content of the food.

For more complete discussions of aspects of energetics and growth in salmonids and fishes in general, the following are suggested: Warren and Davis (1967), Carlander (1969), Weatherley (1972), Brett (1979), Brett and Groves (1979), Ricker (1979), Summerfelt and Hall (1987), and Busacker et al. (1990) (*see also* Chapter 7).

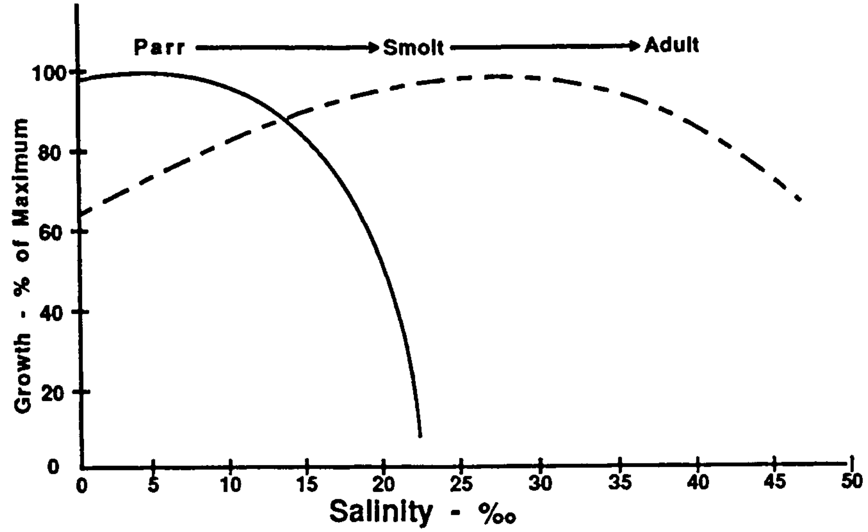

FIGURE 9. The effect of salinity on growth of parr (solid line), and post-smolt and adult (broken line) stages of anadromous salmonids (simplified from Brett 1979).

## REPRODUCTIVE BIOLOGY

### *Characteristics of Mature Adults*

The typical adult length, longevity, age at first maturity, approximate fecundity, egg size, and thermal requirements for spawning and incubation of major salmonid species are summarized in Tables 10 and 11. However, readers should be aware that wide variations exist in these "typical" values resulting from both genetic make-up and environmental conditions. Moreover, notable differences occur among various life history and reproductive traits between anadromous and non-anadromous forms of a particular species. Many of these differences relate to differences in adult size (*see* Gross 1987).

### *Environmental Factors*

Gametogenesis (i.e., spermatogenesis and oogenesis) and the events associated with spawning, which include final maturation of oocytes, and ovulation and spermiation, are affected by many factors. The sequence and timing of these factors is important, as

TABLE 10

Typical values[a] for selected size, age, and reproductive characteristics of important salmonids (from Berg 1948; Tanaka 1965; Carlander 1969; Wheeler 1969; McPhail and Lindsey 1970; Hart 1973; Scott and Crossman 1973; Martin and Olver 1980; Holčík et al. 1988; Scott and Scott 1988; Goetz 1989; Jatteau 1991; Meehan and Bjornn 1991).

| **Genus and species** | **Adult length (mm)** | **Longevity (yr)** | **Age of first maturity (yr)** | **Approx. fecundity** | **Egg size (mm)** |
|---|---|---|---|---|---|
| ***Oncorhynchus*** | | | | | |
| pink salmon | 610 | 2 | 2 | 1500-1900 | 6 |
| chum salmon | 635 | 3-4 | 3-4 | 2400-3100 | 5.1-5.9 |
| coho salmon | 457-610 | 3-4 | 3-4 | 2100-2800 | 4.5-6.9 |
| masu salmon | 400-700 | 3-4 | 3-4 | 2000-4000 | 7 |
| sockeye salmon | 610 | 4-5 | 4-5 | ≈3700 | 4.5-5.0 |
| (kokanee) | 203-229 | 4 | | | |
| chinook salmon | 839-915 | 4-5 | 4-5 | ≈4800 | 6-7 |
| cutthroat trout | 305-381 | 4-7 | 2-4 | 1100-1700 | 4.3-5.1 |
| rainbow trout | 305-457 | 3-5 | 3-5 | 4100-4400 | 3-5 |
| (steelhead) | 508-762 | 6-8[b] | | | |
| ***Salmo*** | | | | | |
| Atlantic salmon | 457 | 4-9 | 3-7 | 1550-1750/kg | 5-7 |
| brown trout | 406 | 5-16 | 4-5 | 1500-4000 | 4-5 |
| ***Salvelinus*** | | | | | |
| Arctic charr (N. America) | 351-487 | 15-20 | 7-12 | 3000-5000 | 4-5 |
| bull trout | 300-750 | 10-12 | 3-6 | 1300-8800 | 4.5-6.2 |
| brook trout | 254-305 | 4-6 | 2-3 | 500-3000 | 3.5-5 |
| Dolly Varden | 457-610 | 10-12 | 3-6 | 1300-3400 | 4.5-5.5 |
| lake trout | 381-508 | 12-23 | 6-7 | 1000->10000[c] | 5-6 |

TABLE 10 cont'd.

| Genus and species | Adult length (mm) | Longevity (yr) | Age of first maturity (yr) | Approx. fecundity | Egg size (mm) |
|---|---|---|---|---|---|
| *Hucho* | | | | | |
| huchen | 700-1000 | 20[d] | 3-5 | 1000-1600/kg | 3.6-6.0 |

[a] The values are very general only; in actuality, length, age, maturity, fecundity, and, to a certain extent, egg size are highly variable with stock and size of the fish and their environment.

[b] Also Great Lakes (North America) populations.

[c] Extremely wide ranges in fecundity reported from published literature.

[d] Oldest recorded but estimated to be much higher (Holčík et al. 1988).

spawning (particularly ovulatory) failure may result if environmental cues are not present or properly synchronized.

Environmental factors affecting reproduction are usually discussed as: proximate factors, which stimulate gonadal development and breeding activities; zeitgebers, which entrain endogenous rhythms; and ultimate factors, such as sufficient food and favourable habitat conditions, which affect the success and continuation of the species. Environmental influences on the stages of reproduction, including both gonadal recrudescence and regression, include: photoperiod, temperature, seasonal variation, endogenous rhythms, water current, pH, barometric pressure, social factors, and stress, among others. Readers are advised to consult comprehensive reviews on this subject, including those of de Vlaming (1974), Sundararaj (1981), and Lam (1983). More complete information on spawning and incubation temperatures, water depths, velocities, redd characteristics, and other natural habitat requirements in general are documented by Bjornn and Reiser (1991) (*see also* Chapter 3).

### *Hormonal Control*

Breeding cycles of salmonids are clearly correlated with elevated levels of gonadal steroids (*see* Liley and Stacey 1983). The predominant gonadal steroid hormones involved in maturation of male salmonids are testosterone (T) and 11-ketotestosterone (11-kT). Recent evidence suggests that 11-kT is the functional form in salmonids and elevated levels are associated with spermiation and the onset of secondary sexual characteristics, such as colour development, appearance of a kype, and aggressive behavi-

TABLE 11

Natural spawning and egg incubation temperatures (°C), and daily temperature units (DTU°C) to hatching for eggs of selected salmonid species. DTU values are from Piper et al. (1982) except for cutthroat trout (Liknes and Graham 1988), masu salmon (Tanaka 1965), Arctic charr (Swift 1965), and huchen (Holčík et al. 1988). Mean DTU values ([a]) are calculated from values provided for different temperatures in those references cited (*see* Table 12).

| Species | Spawning | Incubation | Reference | DTU |
|---|---|---|---|---|
| ***Oncorhynchus*** | | | | |
| pink salmon | 5-19 | 8-10.5 | Raleigh & Nelson (1985) | 500 |
| chum salmon | 7.2-12.8 | >4.5 | Hale et al. (1985) | 611 |
| coho salmon | 7-13 | | Piper et al. (1982) | 417 |
| | 4.4-9.4 | 4.4-13.3 | McMahon (1983) | |
| masu salmon | 5-12 | 7-11 | Tanaka (1965) | 494[a] |
| sockeye salmon | | 7-12 | Piper et al. (1982) | |
| chinook salmon | | 7-13 | Piper et al. (1982) | 417 |
| | 4.4-18 | 4.4-9.4 | Raleigh et al. (1986*a*) | |
| | 5.6-13.9 | 5.8-14.2 | Allen & Hassler (1986) | |
| cutthroat trout | | ≈10 | Hickman & Raleigh (1982) | 310 |
| rainbow trout | 4-19 | | Elliot (1981) | 323[a] |
| | 6-8 | | Alabaster & Lloyd (1982) | |
| | 10-13 | | Piper et al. (1982) | |
| | | 7-12 | Raleigh et al. (1984) | |
| steelhead | | 3.9-9.4 | Pauley et al. (1986) | |
| ***Salmo*** | | | | |
| Atlantic salmon | 0-8 | | Elliot (1981) | |
| | 1-6 | | Alabaster & Lloyd (1982) | |
| | 6-10 | | Piper et al. (1982) | |
| brown trout | 1-10 | | Elliot (1981) | 394[a] |
| | 2-6 | | Alabaster & Lloyd (1982) | |
| | 9-13 | | Piper et al. (1982) | |
| | 7-9 | 2-13 | Raleigh et al. (1986*b*) | |
| ***Salvelinus*** | | | | |
| Arctic charr | 0.5-7 | <7 | Johnson (1980) | 425[a] |
| | 3-15 | | Elliot (1981) | |

TABLE 11 cont'd.

| Species | Spawning | Incubation | Reference | DTU |
|---|---|---|---|---|
| ***Salvelinus* cont'd.** | | | | |
| Arctic charr cont'd. | | 3.5-8 | Steiner (1984) | |
| brook trout | <9 | ≈6 | Power (1980) | 416[a] |
| | 2-16 | | Elliot (1981) | |
| | 7-13 | | Piper et al. (1982) | |
| | 4.5-10 | | Raleigh (1982) | |
| lake trout | ≈10 | | Martin and Olver (1980) | 440[a] |
| | 9-11 | | Piper et al. (1982) | |
| | 4.5-14 | | Marcus et al. (1984) | |
| bull trout | <9 | 2-5 | Goetz (1989) | |
| ***Hucho*** | | | | |
| huchen | 6-10 | 5-12 | Holčík et al. (1988) | 272[a] |
| | 8-10 | | Jatteau (1991) | |

our (Idler et al. 1971; Sangalang and Freeman 1974; Scott et al. 1980). The general trend in salmonids is for T and 11-kT to increase during the period of spermatogenesis and spermiation to peak levels of up to 100 - 150 ng/ml (Scott and Sumpter 1983).

Other steroids present in male salmonids during maturation include progestins, estrogens, and corticosteroids (*see* Fostier et al. 1983; Liley and Stacey 1983). The progestin, 17$\alpha$-hydroxy-20ß-dihydroprogesterone (17$\alpha$20ß-DHP) increases to about 30 - 40 ng/ml during maturation and changes in levels correspond with sperm production (Scott and Sumpter 1983).

In female salmonids, major steroid hormones associated with maturation are 17ß-estradiol ($E_2$), T, and 17$\alpha$20ß-DHP. Fluctuations in these hormones during the maturation period are extensive and exhibit clear patterns. During egg development, plasma $E_2$ levels increase from low levels to about 10 - 20 ng/ml (Fostier et al. 1983) or as high as 40 - 50 ng/ml (Scott and Sumpter 1983). However, before spawning, $E_2$ levels decline to pre-vitellogenic levels as 17$\alpha$20ß-DHP levels increase. As circulating $E_2$ acts to suppress pituitary release of gonadotropins (GTH) by negative feedback, a drop in $E_2$ allows for increased secretion of GTH necessary to stimulate final ovulation.

Testosterone is a major reproductive steroid in female salmonids and may function to affect GTH secretion, stimulate behaviour, or serve as a precursor for other steroids

(Liley and Stacey 1983). Levels of T in females may be high, up to 200 - 400 ng/ml, during maturation and are often higher in female than male salmonids (Fostier et al. 1983; Scott and Sumpter 1983). The maturational steroid in salmonids, 17$\alpha$20ß-DHP, rises to a peak [e.g., >400 ng/ml (Scott and Sumpter 1983)] and then declines relatively quickly shortly before ovulation. The rise in 17$\alpha$20ß-DHP is associated with the decrease in $E_2$ and the increase in GTH during oocyte maturation. One role of 17$\alpha$20ß-DHP apparently is to induce germinal vesicle breakdown subsequent to its migration from the centre to the periphery of the oocyte (Goetz 1983).

Corticosteroids, notably cortisol, are elevated during adult freshwater migrations of anadromous salmonids. Cortisol is considered a catabolic steroid and increases in titre are often associated with stress (Barton and Iwama 1991; Pickering 1992), but its precise role during maturation of salmonids is not clear.

### *Vitellogenesis*

Vitellogenesis is a sequential process incorporating the following events: (1) induction of vitellogenin synthesis and its release into circulation, (2) transport of vitellogenin in the blood stream to the target tissue, (3) uptake of vitellogenin by the growing oocytes, and (4) the conversion of vitellogenin into storage forms (Ho 1987). Vitellogenesis takes place in all oviparous vertebrates, including salmonid fishes. Vitellogenin is the precursor protein used for production of egg yolk and consists of a relatively large protein chain base as well as lipids, carbohydrates, phosphate groups, and mineral salts. Vitellogenin acts as the carrier molecule for materials for the developing egg. Fish display relatively high variability in vitellogenin features compared with other vertebrates and even within salmonids, molecular weight may range from 342 to 600 kDA (Mommsen and Walsh 1988).

The site of vitellogenesis is the liver where synthesis takes place in the hepatocytes. Gonadotropins, released by the pituitary gland, stimulate the gonadal tissue of the maturing female fish to synthesize and release $E_2$, which is carried in circulation to the liver. After $E_2$ binds with a specific receptor within the target cell (i.e., the hepatocyte), the $E_2$-receptor complex stimulates the synthesis of the vitellogenin protein.

Vitellogenin is transported in the plasma to the ovary where it is taken up quickly by the developing oocytes after binding with specific receptors on the cell membrane. The vitellogenin is then "processed" into its constituent egg yolk components as it is translocated from the surface to the appropriate yolk deposition sites within the egg.

Products of vitellogenin breakdown, namely phosvitin and lipovitellin, are required, along with other compounds, for embryonic development and to provide energy during this critical life stage. Other compounds accumulated by the oocytes are glycogen, carotenoids, lectins, sialoglycoproteins, and wax and sterol esters. For a comprehensive review on the process of vitellogenesis and its role in fish egg development, see Ho (1987) and Mommsen and Walsh (1988).

### *Characteristics of Sperm and Eggs*

*Sperm.* In teleost fishes, the testes are characterized by the presence of tubules within which the germ cells develop in cysts, or follicles, as soon as spermatogenesis begins (Roosen-Runge 1977). The spermatocyte of teleosts, like most organisms, consists of head, "middlepiece", and tail portions. Most of the head portion is occupied by the nucleus, which has a DNA content of about 48% in salmonids (Ginzburg 1968). In the testes, there is an apparent accumulation of high-nitrogen content amino acids during maturation that may be related to the synthesis of nucleic acids, as this change only occurs during spermatogenesis (Love 1970). The spermatocyte tail is formed by an axial filament surrounded by a cytoplasmic layer. The tail is about 42% protein, 32% lecithin, and 26% lipid material; no nucleic acids are present in the tail (Ginzburg 1968). The entire spermatocyte is covered by an outer membrane, or plasmalemma. Recent examination of salmonid (e.g., rainbow trout) sperm membrane indicated the presence of over 50 proteins and seven classes of phospholipids (Labbé and Loir 1991). Salmonid spermatocytes are about 20 - 60 $\mu$m in length, depending on species, with head dimensions of 2.0 - 4.5 $\mu$m long and 1.5 - 4.0 $\mu$m wide (Ginzburg 1968).

Piironen and Hyvärinen (1983) measured the composition of milt in rainbow trout, brown trout, and Atlantic salmon; mean values ranged from 17.8 to 33.5% for spermatocrit (volume of sperm in milt) and 9.2 to 14.1 x $10^9$/ml for sperm density, and from 4.7 to 17.8 mg/dl for glucose, 0.20 to 0.33 mg/dl for fructose, 5.5 to 39.7 mg/dl for citric acid, 0.56 to 2.3 mg/dl for glycerol, and 10.4 to 39.4 mg/dl for total lipids in seminal plasma.

*Egg.* In salmonids generally, primary oocytes begin to form in the ovaries when females are 4 - 6 months old (Scott 1990). The second stage is the development of cortical alveoli in some of the primary oocytes. Cortical alveoli are embedded in the protoplasmic layer of unfertilized eggs and after fertilization, break down to release colloids, which become a component of the fluid that fills the perivitelline space (Yamamoto 1961). When the female becomes mature, yolk globules start to appear in the egg cytoplasm and the oocyte begins a period of exponential growth that culminates with ovulation. The development of salmonid eggs occurs synchronously.

Salmonids, compared with most teleosts, have relatively large eggs when developed, ranging from about 3 to 7 mm in diameter (Table 10). A number of factors affect egg size including fecundity, parental size, stock, and season (Blaxter 1988). The bulk of the egg volume is yolk, which is retained within the vitelline membrane (Figure 10). Between the yolk and the shell, or chorion, is the fluid-filled perivitelline space, which allows the yolk and germinal disc (Figure 10) to float freely within the egg. The micropyle, an opening at the animal pole in the chorion (Figure 10), provides entrance for the sperm at fertilization.

Salmonid eggs are about two-thirds water, as their dry weight is about 34% of wet weight (Smith 1957). Most of the egg is comprised of protein (20% of wet weight), the major component of yolk (*see* Vitellogenesis) and the source for tissue elaboration. Other components of the egg (e.g., rainbow trout) are: oil, 4%; phospholipid, 4%; carbohydrate, <1%; and ash, 1.3%, of wet weight (Smith 1957). Srivastava and Brown (1991) found that wild salmonid eggs (e.g., Atlantic salmon) contained higher amounts of protein, lipids, and carbohydrate, and, thus, had a higher energy content than did eggs from hatchery stocks.

Fecundity, or the number of eggs produced by a female in a single year, is variable among species (Table 10). Fecundity also varies considerably within a species due to parental size or age, stock or geographic location, and environmental factors such as temperature and diet (Blaxter 1988). For example, fecundity increases with fish length (or weight or age) according to the relationship:

$$F = aL^b \tag{15}$$

where F = fecundity, L = length, and a and b are constants (Blaxter 1969).

### *Fertilization and Embryology*

Fertilization in salmonids is monospermic. Under natural conditions, the volume of milt ejected at a single time can range between 3.5 and 20 ml and contain $10 - 25 \times 10^9$ spermatozoa per ml (Ginzburg 1968). At fertilization, a low-molecular weight substance apparently induces sperm aggregation in the vicinity of the micropyle (Yamamoto 1961). However, the micropyle is sufficiently narrow to allow only one sperm cell to enter the egg at a time. Specific surface sites (antigens) in the head region of spermatozoa appear to be necessary for the interaction between gametes so that fertilization can occur (Beck et al. 1992).

After fertilization, the chorion and cytoplasm of the egg separate and a plug is formed in the micropyle to prevent further entry by sperm (Blaxter 1969). The separation of the egg cortex and chorion results in the formation of the perivitelline space. This space is filled with a fluid containing colloidal material, the perivitelline fluid, which maintains the proper osmotic tension within the egg. Hardening of the chorion itself also protects the embryo during the early stages of development.

These changes take place during the "water-hardening" process, which takes about 30 to 90 minutes in salmonids, depending on temperature (Piper et al. 1982). Initially at

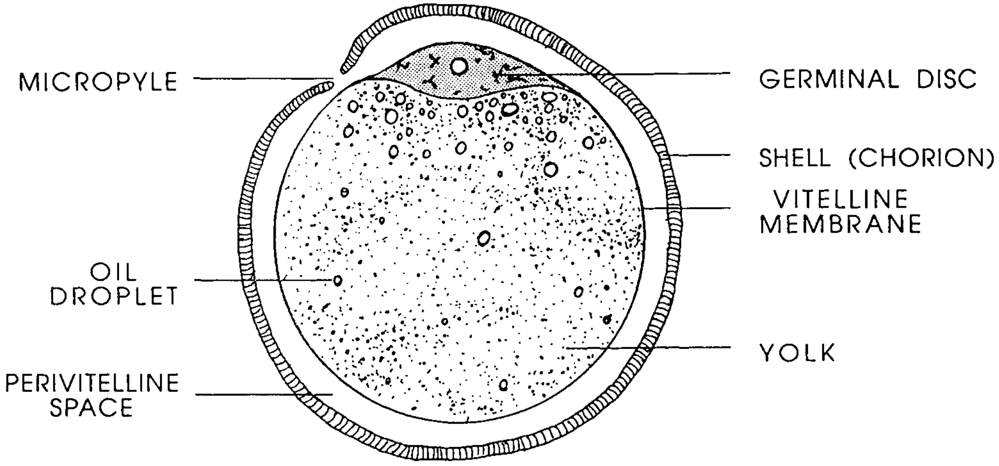

FIGURE 10. Cross-sectional diagram of a salmonid egg (redrawn from Piper et al. 1982).

fertilization, the chorion is permeable to water. The colloid material within the perivitelline space creates an osmotic gradient that results in water being drawn in until the egg becomes turgid. Once the chorion hardens, however, further movement of water into the egg is prevented.

Cleavage in salmonids is meroblastic; that is, early cell division results in the formation of a blastodermal cap at the animal pole of the egg (Blaxter 1969). The early period of development until the blastopore stage is complete is an extremely sensitive one for salmonid eggs (Leitritz and Lewis 1980). In accordance with a model developed for embryonic growth in general, salmonid embryos grow as described in the following equation:

$$w = a(t-n)^b \qquad (16)$$

where $w$ = weight, $t$ = age, $n$ = time to establishment of the embryonic axis, $a$ = intercept of line with the weight axis, and $b$ = slope of the line (modified from Hayes 1949).

The growth and development of salmonid eggs are temperature dependent and require a certain number of thermal units, a function of temperature and time. Particular caution is advised when interpreting daily temperature units (DTU) required for egg incubation (Table 11). The concept that "degree-days" is a constant function regardless of temperature is not valid as this function also varies with temperature (Table 12). Although the simple degree-day model is useful, other models have been developed that predict egg incubation time relative to temperature more accurately (e.g., Alderdice and Velsen 1978; *see* Chapter 5).

It is beyond the scope of this chapter to provide a detailed description of salmonid embryology, but simple diagrams outlining the progression of early salmonid development are provided in Leitritz and Lewis (1980) and Piper et al. (1982). Excellent descriptions, with detailed diagrams and plates, describing the embryonic stages of a number of *Salvelinus* species are presented by E. Balon and other authors in Balon (1980). Vernier (1969) and Holčík et al. (1988) present similarly detailed descriptions of rainbow trout and huchen embryology, respectively. Readers interested in this early phase of salmonid ontogeny are encouraged to consult those references.

### *Larval Biology*

Critical periods for larval fish are hatching, first feeding, early respiration, swim-up, and later metamorphosis (e.g., smoltification) (Blaxter 1988). After hatching, the larvae, or alevins, undergo a number of metamorphoses as they develop. Salmonid developmental changes are not as marked as in other species, but they include development of scales, pigmentation, and the appearance of haemoglobins.

Salmonid larvae are relatively large compared with other teleosts and gill development is rudimentary. Like most teleosts, initial post-hatching respiration is cutaneous. As the larvae develop, the $O_2$ requirement per unit weight declines with increasing body length. Body surface area per unit weight declines more quickly, however, so that cutaneous respiration is insufficient to satisfy larval requirements. Thus, some extra $O_2$–carrying capacity of the blood, e.g., via haemoglobin, is essential at this stage. Although the blood of many species' larvae does not contain much haemoglobin until later metamorphosis, haemoglobin is present and the blood may appear pink at hatching (Blaxter 1988).

As the alevin develops, the yolk sac decreases in size as the yolk materials are absorbed. Efficiency of yolk utilization (i.e., percentage of yolk that is used for tissue elaboration rather than maintenance) generally ranges from 40 to 70% for fish and depends on temperature, original egg weight, light conditions, contour of the substrate, and water turnover (Blaxter 1969). When the yolk sac is almost completely absorbed, alevins begin to feed exogenously; this is the developmental stage commonly referred to as "swim-up fry". Unlike most species, salmonids have a functional stomach present before external feeding begins (Blaxter 1988). Food taken at first feeding is related to the gape of the mouth, but also to prey abundance, size, availability, and mobility. Of all factors, prey abundance appears to be the principle one influencing food selection in

**TABLE 12**

Daily temperature units (DTU°C) to hatching determined for selected salmonid species at different temperatures (data from Swift 1965; Tanaka 1965; Piper et al. 1982; Holčík et al. 1988) (mean values are reported in Table 11).

| Temperature (°C) | Rainbow trout | Brown trout | Brook trout | Lake trout | Arctic charr | Masu salmon | Huchen |
|---|---|---|---|---|---|---|---|
| 1.7 | | 260 | 240 | 270 | | | |
| 4.0 | | | | | 383 | | 310 |
| 4.4 | 356 | 444 | 458 | 480 | | | |
| 6.0 | | | | | 450 | 525 | 300 |
| 7.2 | 347 | 462 | 491 | 520 | | | |
| 8.0 | | | | | 434 | 460 | 282 |
| 10.0 | 310 | 410 | 444 | 490 | 430 | 500 | 263 |
| 12.0 | | | | | 426 | | 245 |
| 12.8 | 307 | | 447 | | | | |
| 14.0 | | | | | | 490 | 230 |
| 15.6 | 296 | | | | | | |

larval fish (Noakes and Godin 1988). The life histories of salmonid fishes beyond the larval stage are discussed in detail in the next chapter (Chapter 3).

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## Chapter 3

# SALMONID LIFE HISTORIES

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## INTRODUCTION

The salmonids (family Salmonidae, subfamily Salmoninae) are a group of teleost fish species that are naturally distributed in the cold temperate waters of the Northern Hemisphere. They spawn in or on gravel beds in rivers or lake shores where the eggs incubate under the protection of the gravel environment. The young emerge from the gravel as fry ready to eat and migrate. Many of the salmonids complete their life cycle in fresh water, whereas others are anadromous and spend part of their growing phase in salt water. When mature, most return to their ancestral breeding grounds to spawn.

Salmonids are generally large fish and are abundant and easy to catch. They have important commercial and sport value and are a significant food source for aboriginal people. Their simple requirements for incubation under artificial conditions has made it possible to introduce these fish to many new areas in the world, wherever waters were suitable for their development and survival. Also, new understanding of the biological requirements of these fish and refinements of incubation and rearing methods have opened important possibilities for salmonid ranching and farming.

This chapter describes the life histories of 15 important species of salmonids of the genera *Oncorhynchus* (Pacific salmon and Pacific trouts[1]), *Salmo* (trouts or Atlantic trouts), and *Salvelinus* (charrs or chars) (Table 1).

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[1] On the basis of evolutionary information presented by Smith and Stearley (1989), the rainbow and cutthroat trouts, or Pacific trouts, are included in the genus *Oncorhynchus*.

TABLE 1

Species of salmonids important for aquaculture described in detail in this chapter (alphabetical by species; *see* Chapter 2, Table 1 for other common names and other lesser known species).

| | |
|---|---|
| ***Oncorhynchus* spp.** | **Pacific salmon and Pacific trouts** |
| *O. clarki* | cutthroat trout |
| *O. gorbuscha* | pink salmon |
| *O. keta* | chum salmon |
| *O. kisutch* | coho salmon |
| *O. masou* | masu salmon |
| *O. mykiss* | rainbow trout and steelhead |
| *O. nerka* | sockeye salmon |
| *O. rhodurus* | amago salmon |
| *O. tshawytscha* | chinook salmon |
| ***Salmo* spp.** | **Trouts** |
| *S. salar* | Atlantic salmon |
| *S. trutta* | brown trout or sea trout |
| ***Salvelinus* spp.** | **Charrs** |
| *S. alpinus* | Arctic charr |
| *S. fontinalis* | brook charr or brook trout |
| *S. malma* | Dolly Varden charr |
| *S. namaycush* | lake charr or lake trout |

## ORIGIN OF THE SALMONIDS

Salmonids appear to have developed during the Mesozoic Era and were probably already distributed over the Northern Hemisphere land masses by the early Tertiary Period. A fossil find from British Columbia indicates that a salmon-like fish (*Eosalmon driftwoodensis*) existed nearly 50 million years ago (Wilson 1977). The oldest *Salvelinus* fossil was found in northern Nevada and is at least 10 million years old (Cavender 1986). By that time divergence and spread of the major evolutionary groups of charrs had apparently already taken place. Divergence of an ancient *Salmo*-type ancestor into an Atlantic Ocean group (*Salmo*) and a Pacific Ocean group (*Oncorhynchus*) may have occurred about 15 million years ago by mid-Miocene times (Behnke 1992). Thus, the basic pattern of the present geographic distribution of salmonids may have taken place millions of years ago, before the first ice age. This distribution has been influenced and modified by the glacial events during the Pleistocene.

Arguments have been made for both a freshwater and a saltwater origin of salmonids by, for example, Neave (1958) and Thorpe (1982), respectively. Tchernavin (1939) also favoured a freshwater origin of the salmonids. He assumed that the ancestors of Salmonidae were small brightly coloured fishes living in cool streams and lakes of the Northern Hemisphere, and that the diversity of regions over which they spread favoured the formation of many species. Gaps in the geographic distribution that exist at present, as for example the lack of Pacific trouts in Siberia, could be due to extermination as a result of advancement of ice southward during the last glacial time and scarcity of food. Some Salmonidae could have acquired migratory habits by descending to the sea from the frozen rivers in winter in order to feed and some migratory forms could have gradually developed into different species. Whatever the actual evolutionary routes, use of both the freshwater and oceanic environments by many species of salmonids implies important biological adaptations that characterize their life histories (Hoar 1976).

## GENERAL LIFE HISTORY PATTERN

The life histories of salmonids have a common, easily recognizable pattern that includes a number of important biological adaptations. Salmonids are powerful swimmers with sleek, torpedo-shaped bodies and large caudal fins, and they are well equipped, anatomically and physiologically, to perform long journeys in both fresh- and saltwater environments. They are very well equipped to manoeuvre in flowing water and jump waterfalls of up to 3-4 m high.

Salmonids spawn in fresh water and many complete their whole life cycle in the freshwater environment. Others are anadromous and spend part of their lives in salt water and return to fresh water for spawning. Some species have both anadromous and freshwater forms. Among the freshwater forms are sub-populations that move between tributary and main river stems, between river and lake, or spend their whole lives in a particular stream or lake. Spawning generally occurs in flowing water of both mainstem rivers or tributaries, depending on the species. Some species, or sub-populations of species, may spawn in standing water in gravel or rock beds on shoals or along lake shores. Spawning areas are generally characterized by water percolating up through the gravel or stones.

When the time approaches for reproduction, which is generally spring for the Pacific trouts and autumn for all the other salmonid species, the maturing adults move to the spawning grounds. Often extensive migrations are involved and invariably the fish return to the river or lake of origin. Most salmonids change their body shape when they mature by developing secondary sexual characteristics, such as humps and kypes on either the upper (Pacific salmons) (Figure 1) or lower jaw (Pacific trouts, Atlantic salmon, and brown trout). Colouration changes from the darker backs and silvery lateral sides that they have during the feeding phase of their lives, to shades of red, orange, yellow, green, purple, brown, black, and white. Often the underparts are darker in colouration than the upper parts. When spawning time approaches the fish change from a countershading and blending kind of colouration and pattern to one with strong advertising significance that emphasizes roundness in combination with bright colours (Figure 1). Some species lose their scales when they reach maturity (e.g., Pacific salmon) allowing the colour pigments in the skin, which are greatly intensified during this period, to be displayed.

Spawning behaviour in salmonids consists of nest site selection, nest territory defence, nest construction, courtship, mating, covering of the eggs, and redd defence. The female selects the nesting site and digs the nest. The male courts her during the nest preparation process and protects the nest territory from intruding males. When the nest is completed, both female and male crouch down into the nest pocket and release eggs and sperm. Immediately after the eggs have been laid the female closes the nest with gravel and in the process starts to dig the new nest in front of the previous one. When all the eggs have been deposited in a series of nest pockets, the female covers the nesting area with a gravel mound, called a redd, and protects the site against other females.

In Pacific salmon, males and females generally die after spawning[2], whereas in Pacific trouts, Atlantic salmon, brown trout, and charr species, a portion or most of the population may survive. The survivors move away after spawning to feeding areas, to return one or more times during the following years to repeat the process. Thus, there is no parental protection of the brood during incubation and larval development in salmonids.

The eggs develop in the gravel over the winter and are well protected from floods, ice conditions, and predators. This protective advantage from elements and predators is supported by the relatively low fecundity of less than 100 to several thousands of eggs. The large yolk volume carries the animal through a period when food is generally limiting.

The rate of development during incubation of the eggs is dependent on water temperature and genetic characteristics related to environmental conditions of the specific sub-population. The higher the temperature the faster the development. Thus, embryo growth proceeds more slowly for autumn spawners, whose eggs develop during the winter under cold water conditions, than for spring spawners, whose eggs develop as water temperatures are increasing during late spring and early summer.

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[2] Some masu and amago salmon, especially the females, are known to survive spawning and may reproduce several times before dying (Kato 1991).

When 10-20% of the yolk has been utilized during embryonic development, the alevins, young fish with a yolk sac attached to their belly, hatch from eggs for further development in the protective gravel environment. For autumn-spawning species, alevin development can take several months, whereas for spring-spawning species, this development can take place in a few weeks. The alevins generally stay in the gravel or crevices until the yolk sac has been absorbed and then emerge as fry, primarily during the early hours of darkness. Fry emergence for both the autumn and spring spawners takes place within narrow time windows, despite the fact that the timing of entry into the river and spawning of the mature adults can be spread out over a considerable period of time. According to Brannon (1987), the emergence time of fry is the major evolutionary influence that established spawning time. Brannon (1987) concluded that, "Because the best emergence time occurs within a relatively short time interval in a given stream system, and the length of the incubation period is determined by the respective temperature regime, the appropriate time for spawning to take place becomes relatively fixed for each population. Spawning time, therefore, is a genetic characteristic of the stock which has been selected to assure optimum emergence timing under the full range of environmental conditions that pertain to each habitat".

Upon emergence, fry may stay in the area of the spawning grounds, but in most cases move downstream, either to the main stem of the river, to a lake, or to the sea. In some instances the fry will move upstream into a lake when spawning has occurred in an outlet stream. Newly emerged fry tend to hide during the day among rocks and gravel and only move into open water during the night. After a few days this night-active behaviour changes to greater activity during daylight hours. For most salmonid juveniles, dusk and dawn are important feeding times; some, however, may feed all day long. As food requirements change the juvenile and immature salmonids shift to different habitats. Some of these habitat changes may be accomplished by short journeys, but in many cases they include extensive migratory movements that have given the salmonids the reputation as profound long-distance travellers (Groot 1982).

In Pacific salmon, steelhead, and Atlantic salmon, the juveniles migrate to salt water after shorter or longer periods in fresh water. Most trouts and charrs complete their life cycle in fresh water, although some may enter salt water for varied time periods. In the freshwater environment they may either stay in the river where they were born, enter other parts of the river tributary, or move into lakes.

Pacific salmon juveniles initially migrate along the coasts of the North American and Asian continents before moving offshore to the overwintering grounds (Hartt and Dell 1986). The juveniles of Atlantic salmon and steelhead, which are generally larger than those of Pacific salmon when they enter salt water, appear to move offshore immediately and can travel great distances in the ocean in a matter of a few months (Burgner et al. 1992).

Distribution of Pacific and Atlantic salmon and of steelhead trout in the ocean can be extensive as a result of long-distance migrations, or more limited to near-shore areas, depending on the species or stock. Pacific salmon make north-south movements with the seasonal changes in ocean temperatures during one or more years and then, when they mature, migrate shoreward to their home river to spawn (Groot and Margolis 1991; Welch et al. 1995). The strong tendency of salmonids in general to return to the place of birth for spawning and the resulting geographic and reproductive isolation of the sub-populations have led to the development of many ecological forms during evolution (Thorpe and Mitchell 1981; Behnke 1984, 1992; Groot and Margolis 1991).

Those species that spend a portion of their lives in streams during the juvenile or immature stages have parr marks along their lateral sides (Figure 1). Parr marks act as a disruptive pattern for the body outline and allow the fish to blend into the gravelly or rocky background. Bright patterns occur on the fins, primarily dorsal, ventral, and anal fins, and are used as display signals in agonistic encounters. The fins are folded to hide the display signals at times that it is necessary to conceal.

Movement to open and deep waters of a lake or ocean coincides with a loss of the parr marks and development of a distinct countershading pattern with dark upper-parts and silvery sides and belly (Figure 1). This pattern counteracts the roundness of the body as caused by light shining from above and conceals the fish in the vast greyness of open water. Fish that stay in streams and rivers often retain their parr marks even as mature individuals, as is the case for masu and amago salmon.

Upon reaching maturity, the cycle is completed by the return of the fish to the ancestral breeding grounds to spawn. Total life span among the salmonids is variable and ranges from 2 for pink salmon up to 26 or more years for some charrs (*see* Chapter 2). Salmonids with shorter life spans, such as the Pacific salmons, spawn only once, while those living longer can perform from one to many repeat spawnings during following years.

There is great variation in weight among the salmonids. Growth in open water environments, such as lakes and ocean, is generally greater than in streams. In rivers, salmonids usually do not become larger than a few kilograms, whereas in lake and ocean environments weights of over 50 kg have been recorded for lake charr and chinook salmon. Fecundity can range from 70 eggs per female for small amago salmon to more than 17,000 for large chinook salmon and 21,500 for large lake charr.

On the general theme of spawning in fresh water and movements between breeding and feeding grounds, the Pacific salmon, trouts, and charrs have developed a great variety of life history variations during evolution. These variations, which can vary both among as well as within species from region to region, are closely related to the ecosystems inhabited. The adaptability and developmental plasticity of these fish is

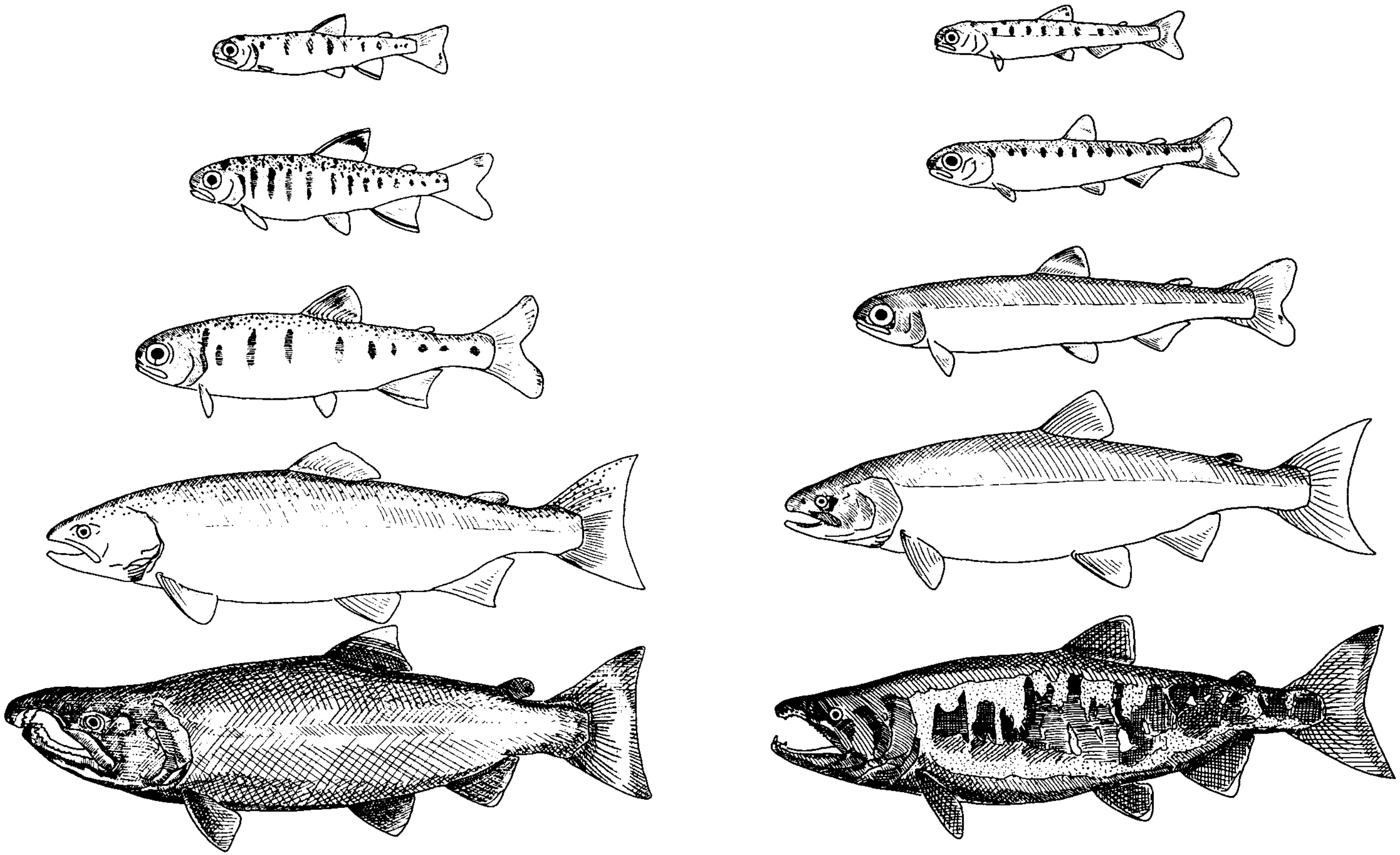

FIGURE 1. Life history stages of coho salmon (left) and chum salmon (right). From top to bottom: fry, fingerling, smolt, ocean phase, and mature adult male.

remarkable and during evolution they have been able to occupy many different fresh water and marine environments.

## EARLY DEVELOPMENT

The early developmental stages are the most sensitive in the life histories of salmonids and are critical with respect to survival. The highest mortality rates during the life cycle occur during these early stages as a result of adverse abiotic (water temperature, dissolved oxygen content, water flow, turbidity, mineral content) and biotic factors (pathogens, parasites, and predators). The developmental stages, including fertilization, incubation, hatching, emergence and alevin and fry stages, are briefly discussed in this section.

### *Fertilization*

Fertilization of eggs takes place as soon as they are released into the nest and must occur within less than 30 s after extrusion because immediately upon entering water the outer egg membrane becomes a tough, elastic, and insoluble protective capsule (Smirnov 1963). The eggs absorb water through microscopically fine channels of about 1 $\mu$m in diameter, called pore canals that run radially through the chorion, and start to swell (Hayes 1949). Water hardening is independent of fertilization and takes about 1 h to complete (Hayes 1949). The fully hardened egg changes from a soft flaccid lump to a hard, more or less round, miniature "strongbox" within which the developing embryo is protected. If pressed between two flat surfaces the hardened egg withstands a force of 10 kg or more before the capsule breaks. This force equals about 80 kg/cm$^2$, or more than 1,100 lb/in$^2$. During water hardening the egg capsule becomes highly adhesive for about 20 minutes, which helps to keep the eggs in the nest pocket until they have been covered by gravel (Sheridan 1960).

### *Embryonic Development*

Following fertilization the egg (now properly called a zygote) goes through a series of cleavages and an embryo begins to form (Smirnov 1963). During the early part of development, cell layers grow from the top of the egg (the germinal disk) and expand over the egg surface, gradually enveloping more and more of the yolk mass (Velsen 1980). The area remaining uncovered is known as the blastopore. At this stage the egg is extremely fragile and susceptible to mechanical shock. Movement in gravel beds due to freshets, or in hatcheries during cleaning operations, inevitably results in high mortality (Jensen and Alderdice 1983). This sensitive condition alters soon after the blastopore closes, a process which coincides with deposition of black pigment inside the retina of the developing eye. The egg is now known as an "eyed-egg" and this is the preferred stage to move eggs over long distances, plant them in gravel boxes or gravel beds, count them mechanically and, generally, manipulate them without causing harmful effects.

In about 7-10 days, depending on temperature, head and body regions are recognizable (Velsen 1980). The embryo can now be seen to move freely inside the capsule, dragging the large enclosed yolk sac along with it. Blood vessels have started to grow out over the surface of the yolk to form a fine network over its entire surface. A single large vein on the left side collects the absorbed foodstuffs from the yolk into the oxygenated blood stream and discharges it in the main posterior vein a short distance below the now actively pumping heart (Velsen 1980).

### *Hatching*

The eyed stage usually lasts for a few weeks. When the larva is ready to hatch it begins to manufacture a proteolytic enzyme in special ectodermal "hatching" glands located on the head (Popov and Zotin 1961). The release of this enzyme into the perivitelline fluid initiates the hatching process by rapidly corroding the capsule wall (Smith 1957). Soon the actively swimming young fish ruptures the remaining shell and bursts out of it. Hatching is an adaptation brought about by physiological requirements of the developing embryo. When the embryo is small, the surface area of the egg is ample for the required exchange of oxygen and metabolic residues between inside and outside across the thickness of the capsule. As the embryo grows the point is reached where the membrane cannot meet the required exchange rates for the embryo's maintenance and development. The "need" to shed the capsule is now obvious and by hatching, the embryo's body and gill surface areas, which are considerably larger than the surface of the egg, come in direct contact with the oxygen-bearing water (Bams 1969).

The efficiency of transport across body and gill surfaces is about twice that of the egg capsule. Therefore, the embryo's need can be met with only half the available oxygen in the medium after hatching. Experiments have shown that by lowering the oxygen content of the surrounding water, larvae can be induced to hatch long before their normal time. Such adverse circumstances cause early release of the hatching enzyme and eggs hatch out within hours (Hayes et al. 1951). The survival value of this control mechanism is self-evident.

### *Alevin*

Immediately following hatching the larva, now known as an alevin, reflects the shape of the egg; the yolk sac is still spherical and the body is bent. Within a day or so the yolk sac becomes elongated and the body straightens. The fish hatches with its inner ear plus otolith fully formed and operational; the alevin responds to gravity and assume a horizontal upright position (Bams 1969).

When alevins develop in gravel, maintaining an upright position presents no problem, because there are many crevices and surfaces available to help maintain the desired position. However, when alevins are raised on a flat surface, as in certain types of hatchery troughs, the round yolk sac prevents them from remaining in the preferred upright position. The alevin keeps falling over and has to swim to regain horizontal balance. Continuously trying to "right" itself can have detrimental effects on further development of the alevin (Bams 1969). This problem can be alleviated by adding pebbles to the bottom of hatching trays, but this is often impractical in large hatchery operations.

Oxygen and temperature are the two most important factors affecting growth and survival of eggs and alevins during the incubational period (Bams 1969). When water flow and oxygen levels are adequate, growth rate is determined by temperature (Alderdice and Velsen 1978; Beacham and Murray 1986; Murray and Beacham 1986). During early life stages tolerance to cold water is less than during the later embryonal or alevin stages, when development will continue even at 0°C. Differences between species and races exist but, in general, the developmental rate is about twice as fast at 5°C as it is at 0°C, and it again doubles at 10°C. The rate at which the embryo reaches different stages during its development is generally expressed in degree-days, which is time in days × temperature (*see also* Chapter 2). The combined effect of faster developmental rates and increasing temperatures in spring and early summer makes it possible for late winter and spring spawners, such as rainbow and cutthroat trout, to meet the spring deadline for emergence of the fry.

During the first few weeks of development, light, especially at shorter wave lengths, can be lethal or damaging to the embryo. After hatching, alevins are negatively phototactic; that is, they move away from light (Fast and Stober 1984). This behaviour is adaptive because protective colouration does not develop until the end of the larval period. The young alevins have a conspicuous bright orange yolk sac and do not have countershading, barred sides, or reflecting pigment in the skin. Protection from predators by stones and darkness is crucial (Salo 1991).

### *Emergence*

When the yolk sac is almost absorbed and the young fish are fully "buttoned-up", they prepare to emerge from the gravel beds. Emergence is facilitated by rising temperatures and primarily occurs at night (Neave 1955; Hoar 1958; McDonald 1960). However, the behaviour patterns for emergence exist at a much earlier age; the fish can be induced to migrate several weeks prematurely by conditions in the gravel unfavourable to existence, such as low oxygen, high carbon dioxide, or the presence of silt (Coburn and McCart 1967; Bams 1969).

Two guiding systems are used by the migrants to move from the dark gravel into the open stream (Bams 1969). The main one is a response to gravity (negative geotaxis). If the pathway upwards through the gravel interstices is blocked, the fry can switch to a second response manoeuvre and orient themselves against the water flow moving upstream (positive rheotaxis). Both mechanisms facilitate egress from a normal gravel bed. The gravity-oriented system guides the fish along the shortest way out to the stream; the upstream-directed system provides a second choice in case the redd is covered with silt or by some impenetrable object. Cases are reported of salmonid fry surfacing in ditches or wells tens of meters away from the spawning area (Bams 1969).

Although it is hard to visualize young salmon digging through the ground for considerable distances, they are well equipped for this specialized behaviour and utilize several different modes of locomotion. Instead of scales, fry have a smooth tough skin covered with mucus. Besides normal swimming, they can swim up a narrow crevice with a fast tail beat of small amplitude. This motion resembles a "trembling" of the caudal fin, without undulation of the body as in normal swimming. Fry can also move like a snake by curving laterally and pressing against the walls on both sides of the body. To back out of an unsuitable passage, the fish can turn in a surprisingly narrow space. It can drop down passively, or actively slide backwards. It can retract its head from too narrow an opening with a slow powerful tail beat aimed towards the head and repeated in succession on alternating sides of the body. A sand barrier on top of the gravel is overcome by butting into it vertically; the sand grains drop past the butting fish, thus gradually opening up a passageway (Bams 1969).

Readiness to emerge from the gravel is facilitated by higher temperatures (Coburn and McCart 1967). Most streams attain their daily minimum temperature at around 0800 h, and reach their maximum about 12 h later. This results in large numbers of fry, which are ready to emerge, congregating near the surface of the gravel before dusk, where they remain as long as light is present (Bams 1969). Once dark, the inhibitory effect of light is removed and the fry proceed with their emergence behaviour. Thus, the typical diel "fry-run" pattern is established; the run starts shortly after dusk and builds up rapidly to its daily maximum as all the accumulated fish emerge. In a few hours, the numbers drop drastically when, presumably, the only fish that emerge are those that have just reached the gravel surface. As the temperature drops during the night, emergence activity decreases and the fry run stops altogether at the onset of dawn (Coburn and McCart 1967; Bams 1969). Different patterns occur in large river systems, where successive spawning areas contribute to more extended and often complex fry emergence activities.

Upon emergence from the gravel the fry immediately try to get to the surface to take in air to inflate the gas or swim bladder (Saunders 1965; Bams 1969; Dill 1982).

The swim bladder of salmonids retains an open duct connecting it to the dorsal wall of the pharynx (*see* Chapter 2). Air bubbles are obtained with sideways snapping motions. The fish then sound several centimetres and "chew" on the bubble with mouth and gill covers tightly closed, which transfers some of the air through the duct leading from the upper part of the gut to the swim bladder. Several attempts are necessary to inflate the bladder to attain neutral buoyancy. There are indications that fry have to learn what the proper level of inflation is, as too much air will cause the fry to swim head down in order not to float to the surface. In a few hours, however, precise balance is achieved and swimming occurs in a horizontal position (Bams 1969; Dill 1982).

During the process of attaining neutral buoyancy, sockeye salmon fry do not respond to stimuli in their surroundings. When they are artificially induced to emerge in daylight, they ignore food, bump into each other, and do not notice possible predators. They are very vulnerable to predators at this stage and emergence in darkness may, therefore, be an adaptation to reduce predation (Bams 1969).

### *Fry Migration*

There are differences in the fry migration patterns of the Pacific salmon species. Pink, chum and sockeye salmon fry normally emerge at night, do not orient themselves to fixed objects (e.g., bottom), immediately proceed to fill their swim bladders with air, float or swim downstream, actively seek the main stream, and surface when carried into slack water (Bams 1969). If dawn arrives before they reach the estuary in the case of pink and chum salmon fry, or lake nursery area in the case of sockeye salmon fry, they hide in the gravel again (Hoar 1958; McDonald 1960). Some fry may stay near shore in shallow and slack water during daylight hours and form aggregations. Here they orient themselves to fixed objects and continue feeding. After nightfall they give up their fixed positions and move on downstream (Hoar 1950, 1958).

Coho, and perhaps chinook salmon as well, appear to be less inhibited by light and may emerge at any time of the day, especially in the later part of the emergence period (Reimers 1971). As stream dwellers they orient themselves towards fixed objects. They may either hold their position or migrate upstream. Their distribution is influenced by territorial behaviour, which limits density in resident areas of the stream. If supply of fry is greater than the carrying capacity of the stream, the excess fry are pushed downstream by aggressive territory holders (Sandercock 1991). A mass migration of hundreds of thousands of coho fry is an annual occurrence in many streams. If this takes place into salt water of more than 20 ‰ salinity, many fry may not survive because their osmoregulatory mechanisms have not developed yet to handle such concentrations (Otto 1971; Sandercock 1991).

In summary, there are developmental differences among and within salmonid species and populations in timing of spawning and rate of incubation (Heard 1991).

However, timing of fry emergence occurs within narrow time windows in spring for most species of salmonids, when sufficient food is available. The trend during evolution appears to have been to adapt spawning time and embryonic development rates to thermal characteristics of individual streams to assure optimum survival opportunities for fry after emergence in streams, lakes, or the ocean (Sheridan 1962; Brannon 1987).

## FEEDING

Salmonids live in two main types of aquatic habitats; running or lotic waters of streams and rivers, and still or lentic waters of lakes and the ocean. In lotic environments they adopt a "sit-and-wait" feeding strategy (Eriksson and Alanärä 1992). This strategy often involves the defence of feeding territories and the fish operate as individuals. The food organisms are delivered as organic drift and are of two types dependent on where they occur, in the water column or in the surface film. Within the water column, food organisms typically consist of aquatic invertebrates, such as insect larvae, zooplankton, fish eggs, and juvenile fish of all sizes. Organisms available in the surface drift include egg-laying, hatching, and terrestrial insects (*see* Chapter 2, Table 7).

The temporal and seasonal availability of food organisms in streams are highly predictable (Hynes 1970; Müller 1978). Aquatic invertebrate drift primarily occurs at night, during spring and early summer, and generally peaks at dawn and dusk. It diminishes during late summer and early autumn and is absent during winter. Surface drift is primarily a day-time phenomenon and is most abundant during summer and early autumn.

The feeding strategies of salmonids in lentic waters of lakes and the ocean are different. Although availability of food may be generally predictable in these waters, that is not the case for their location. Therefore, the fish actively search for food either within a limited home range or over large areas. In contrast to the individualistic "sit-and-wait" foragers, the "search-and-find" foragers often operate in schools or aggregations.

Prey availability in lentic environments is generally highest in summer. Most zooplankton organisms make vertical migrations; they move up to the surface during the dark hours and occupy greater depths during the day. The salmonids in lakes and ocean perform similar diel vertical migrations (*see* Chapter 6).

### *Diel and Seasonal Cycles of Feeding*

Salmonids show pronounced diel and seasonal variations in movement and feeding patterns. They feed when they are active, which is evident from the close correspondence between levels of movement and feeding (Hirata 1973; Eriksson and Alanärä 1992).

Aquatic animals suitable as food for salmonids in both flowing and standing water environments are primarily nocturnal. Both diel and seasonal variations in feeding activity correspond to the availability of feeding organisms.

Figure 2 shows that the diel locomotor activity pattern of Atlantic salmon parr recorded in a laboratory in mid-March peaked around dusk and dawn. This pattern closely matched the diel distribution of organic drift (mayfly larvae) in a stream next to the laboratory at the same time of year (Figure 2). Vertical migration of sockeye salmon juveniles in Babine Lake, British Columbia, showed a similar correspondence with the diel vertical movements of zooplankton food organisms (Johnson 1961). The fish came up to the surface at the same time that the zooplankters arrived and both prey and predators descended to deeper waters again during daylight hours. Stomach analysis indicated that the fish actively fed during the dawn and dusk periods.

Müller (1978) found that the annual activity rhythm of brown trout tested in a laboratory closely corresponded to the seasonal availability of the food organisms (drifting aquatic insect larvae and surface drift of aquatic and terrestrial insects) in a north Swedish stream next to the laboratory (Figure 3). Organic drift, surface drift, and trout activity all peaked during June and July. Metcalfe et al. (1986, 1988) reported that appetite and growth in Atlantic salmon during autumn and spring matched the autumn drop and spring increase in feeding opportunity, respectively.

The temporal pattern of feeding behaviour in salmonids generally shows high feeding activity at dusk and dawn during late autumn, winter, spring, and early summer and more diurnal feeding during late summer and early autumn (Figure 4). In autumn there is a loss in appetite which lasts until early spring. However, there are species and stock differences among the salmonids. For example, Arctic charr and rainbow trout generally feed nocturnally, whereas feeding of Atlantic salmon and sea-run brown trout is more pronounced dawn and dusk, or even diurnally (Eriksson and Alanärä 1992). There are also differences in feeding behaviour between life history phases dependent on physiological stage. For example, Metcalfe et al. (1986, 1988) showed that juvenile Atlantic salmon that will not smolt the following spring (S2 parr) decrease their appetite significantly from July onward. Those parr destined to become smolts the following spring (S1 parr) maintained high food intake in autumn and even sustained food intake and growth over the winter (Figure 5). This difference in feeding behaviour occurred despite the fact that both groups were under similar rearing conditions.

Activity and feeding rhythms of salmonids are often sustained under constant environmental conditions in tests in the laboratory (Eriksson and Alanärä 1992). This indicates that the fish have evolved endogenous physiological timing mechanisms (biological clocks) with rhythms that are similar to light and temperature cycles in the environment. Eriksson and Alanärä (1992) noted that the diel cycles of feeding are not as rigid as the annual cycles. The diel cycles should probably be considered as some kind of a "template" for best opportunities. As long as the fish is rewarded with food at the

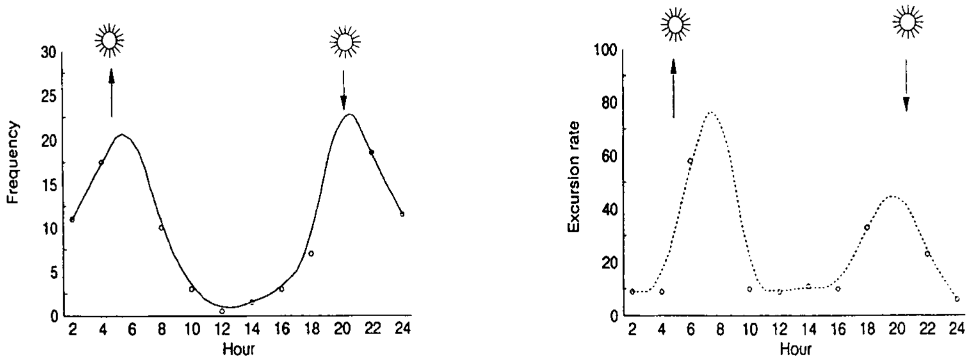

FIGURE 2. Diel organic drift distribution in a north Swedish stream in mid-March (left) and locomotor activity of Atlantic salmon parr in a laboratory at the same stream (right). From Erikkson and Alanärä (1992).

usual dusk and dawn periods, it will stay on this timing. But when food is only available at other times of the day, or when starvation is faced, searching for food will occur at other times of the day.

In addition to diel and annual cycles, coho salmon and rainbow trout also show semi-lunar growth and appetite cycles under constant environmental conditions (Farbridge and Leatherland 1987; Eriksson and Alanärä 1992); this may also be the case for many other salmonids.

### *Feeding Behaviour*

Feeding behaviour is the result of an interaction between stimuli of the prey and the internal state of the animal (Tinbergen 1951). Motivation to eat, which is dependent on appetite and endogenous activity rhythms, determines the strength of the feeding response to stimuli of the prey, such as movement, size, and colour. Many of the feeding responses are innate and can be improved by experience. Fish that are not motivated to feed will ignore even highly favoured food items (Huntingford and Thorpe 1992).

Feeding is a combination of a number of activities that occur in sequence (Figure 6) (Stradmeyer 1992). The activities include:

(1) Orientation — focusing on the prey with eye and head movements
(2) Approach — actively swimming towards the prey
(3) Capture — grabbing the prey with the mouth and testing for texture and taste
(4) Ingestion — eating and swallowing the prey when it passes the test
(5) Spitting — when the prey does not pass the test

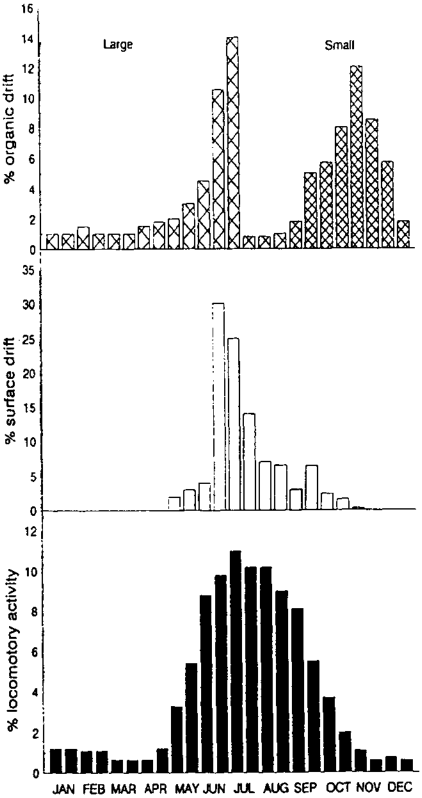

FIGURE 3. Annual patterns of organic and surface drift in a north Swedish stream and the activity distribution of brown trout in a laboratory at the same stream. From Müller (1978).

Each of these activities is elicited by stimuli, such as visual, olfactory, tactile, gustatory, and possibly auditory characteristics of the prey, which act as releasers. The combination of stimuli that elicits certain behaviour activities can be studied by releaser experiments in which specific combinations of potential releasers are presented in choice experiments (Tinbergen 1951). Only a small number of such experiments have been carried out with salmonids with respect to feeding (Stradmeyer 1992).

Visual stimuli of the prey that may play an important role during the orientation, approach, and attack phases include movement, size, shape, colour, and contrast. Movement of prey is a strong releaser of feeding behaviour in many animals. Rainbow trout increase their reactive distance when the prey moves more (Ware 1972), and passively transported particles are less attractive to fish than wild prey that move (Stradmeyer and Thorpe 1987). Atlantic salmon fry prefer copepods to cladocerans at

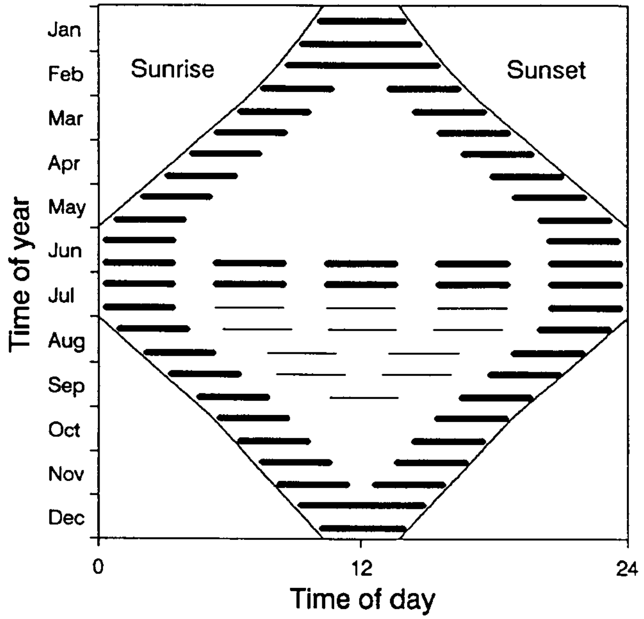

FIGURE 4. The diel and annual excursion rate of brown trout under natural temperature and light conditions at the Arctic circle in Sweden. From Erikkson (1975).

high densities, probably because copepods swim intermittently and stand out against the continuously swimming cladocerans (Browman and Marcotte (1987).

Salmonids are opportunistic feeders and will generally eat what is available in their environment when it is within the preferred size range (R. LeBrasseur, Pacific Biological Station, Nanaimo, B.C., Canada, pers. comm.). However, there is an optimum prey-size preference for each size of fish. Wankowski (1977, 1979) found that prey equal to 2.5% of the fish's own length is preferred by wild and hatchery-reared Atlantic salmon fry.

Although visual cues may be most important for the orientation, approach, and capture phases for the stream-dwelling "sit-and-wait" predator, Eriksson and Alanärä (1992) noted that, "salmonids do not have to rely entirely on vision to feed." They recalled a blind brook charr that cleaned out 30 minnows (*Phoxinus phoxinus*) living in the same tank within 10 days.

After the prey has been captured, tactile and gustatory characteristics of the prey come into play. Stradmeyer (1989) showed that texture of pellets influenced consumption. Soft pellets were ingested twice as often as hard pellets. The pellets were visually identical but differed in texture. The taste and smell characteristics depend on protein, lipid, and carbohydrate body composition of the prey (Higgs et al. 1995). Amino

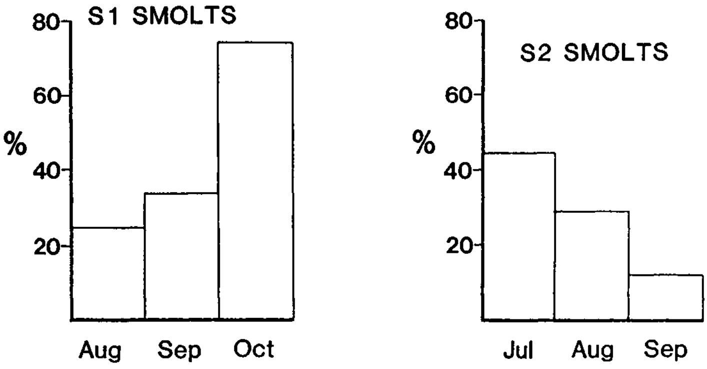

FIGURE 5. Appetite changes of future S1 and S2 Atlantic salmon smolts during their first autumn. From Metcalfe (1992).

acids appear to be most important to stimulate salmon feeding. They attract fish to the food source and encourage tasting (Stradmeyer 1992) and have, therefore, been incorporated in commercial salmon diets (Higgs et al. 1995). Åsgard (1987) found that fish meal and fish oils improve the quality and palatability of pellets. He also noted that the use of squid meal instead of fish meal in rainbow trout and Atlantic salmon diets improved palatability and growth.

## MIGRATION[3]

An important aspect of salmonid biology is that they occupy a variety of habitats during their life cycle; i.e., breeding, incubation, feeding, and overwintering habitats. A series of well-timed and well-directed movements and migrations are performed in shifting between these habitats.

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[3] In this chapter, I have quoted extensively from my own (Groot 1982) publication "Modifications on a theme: a perspective on migratory behaviour of Pacific salmon". To go through the exercise of paraphrasing in order to avoid plagiarism was obviously not applicable.

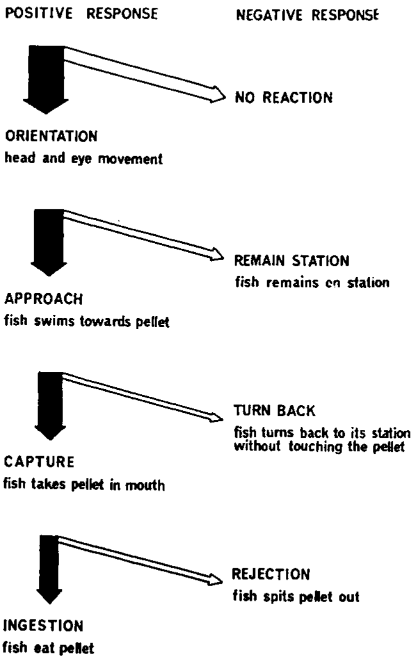


FIGURE 6. Behavioural feeding responses in Atlantic salmon parr used in prey quality tests. Arrow thickness represents the proportion of fish responding to the presentation of a pellet. From Stradmeyer (1989).

The migratory process proceeds in three phases, namely the *preparatory*, *migratory*, and *terminating* phases. During the preparatory phase, environmental factors interact with endogenous rhythms to modify the organism's morphology, physiology, and behaviour to a state of migration readiness, or migration disposition (Baggerman 1960*a*; Hoar 1976; Wedemeyer et al. 1980). In this process the neuro-endocrine system acts as the chemical link between the organism and its environment (Hoar 1965). Preparation is followed by migration, which can be considered a combination of unspecialized locomotory activities and special directing or orientation components (Baggerman 1960*a*). When the migration is completed and the fish have reached the feeding, overwintering, or breeding grounds, it is terminated. Little is known about the termination phase of migration, but it is assumed that both internal rhythms in concert with environmental factors and changes in physiology and behaviour are involved (Baggerman 1960*a*). Because of the extensive research that has been done on Pacific salmon, the following discussion on migration focuses mainly on that salmonid group.

### *Habitat Changes of Pacific Salmon*

Most Pacific salmon spawn in streams, rivers and lakes, and after an early freshwater life of 0-3 yr the juveniles migrate to the ocean. The maturing adults stay in the ocean for one or more years and then return to fresh water to spawn. All Pacific salmon die after one spawning season. Among Pacific salmon, pink salmon have the simplest and shortest life cycle and sockeye and chinook salmon the most complicated (Table 2). The more common habitat changes and migrations of the five Pacific salmon species from western Canada are illustrated in Figures 7 to 11.

Pacific salmon deposit their eggs in gravel beds during spawning in the autumn and the alevins hatch during late autumn or winter, depending on time of spawning and water temperatures during incubation. This is considered the first habitat change (Figures 7 to 11, C1). The period in the gravel can be divided in three phases (Dill 1969). Immediately after hatching alevins move downwards into the gravel, the *downward* phase. This is followed by a period of little movement, the *holding* phase. About a month before emergence the *upward* phase is initiated. The alevins become more lively and start to make progressively greater excursions into the gravel above them. This phase is associated with near absorption of the yolk sac. The upward movement is considered the beginning of habitat change 2 and culminates in spring with the emergence of the fry from the gravel at night.

Pink and chum salmon generally spawn in small and large streams not far from salt water (Heard 1991; Salo 1991). The emerging fry from these two species actively swim or passively float downstream to salt water and often reach the sea during the first night (Figures 7 and 8, C2) (Neave 1955; Hoar 1956, 1958; Heard 1991; Salo 1991). If salt water is not reached during the first night, the fry will hide in the gravel during daylight hours. Migration to the estuary is accomplished by a series of nightly "jumps" in which the fry move as individuals. Upon reaching the estuary, the behaviour changes. The fry become light adapted and start to swim around during daylight hours in schools, actively feeding. During the first few months of saltwater residence, pink and chum fry generally form large aggregations in coastal waters and move rapidly from one area of aggregation to another with pauses in between (Figures 7 and 8, C3) (Parker 1965; Healey 1967). Starting in July and continuing into fall the aggregations break up and there is a general movement offshore and northward along the coastal belt of the Gulf of Alaska (Hartt and Dell 1986) (Figures 7 and 8, C4).

Coho salmon generally spawn in the multitude of small streams along the coast. The fry of most stocks stay in the river of birth after emergence but some move into lakes. The fry that stay in the river move towards the river banks and set up feeding territories. As waters cool during autumn and winter, the juveniles tend to move to river tributaries and overwinter in the deeper parts of the streams, often hiding among leaf debris (Figure 9, C3) (Sandercock 1991). In spring the smolts leave the streams for salt water, primarily at night (Figure 9, C4).

TABLE 2

A summary of the orientation cues and orientation modes (Griffin 1955) that sockeye salmon could potentially use during the different habitat changes they perform during a life cycle.

| | Orientation during migration | | | Griffin's orientation mode |
|---|---|---|---|---|
| | Habitat change | Life stage | Cue | |
| 1. | Egg to gravel | alevin | gravity | I |
| 2. | Gravel to river (downstream) | fry | gravity | I |
| | | | current | I |
| 3. | River to lake (upstream) | fry/fingerling | current | I |
| | | | odour | I |
| | | | landmarks | I |
| | | | celestial | II |
| | | | magnetic | II |
| 4. | Lake nursery area to estuary | smolt | sun | II |
| | | | polarized light | II |
| | | | magnetic | II |
| | | | current | I |
| 5. | Estuary north along shore | immature | electric potential? | I |
| | | | celestial? | II |
| | | | magnetic? | II |
| 6. | Offshore to wintering grounds | immature | electric potential? | I |
| | | | celestial? | II |
| | | | magnetic? | II |
| 7. | Wintering grounds summer feeding grounds | immature | electric potential? | I |
| | | | celestial? | II |
| | | | magnetic? | II |
| 8. | Summer feeding grounds to wintering grounds | immature | coordinate grid system | III |

TABLE 2 cont'd.

| | Orientation During Migration | | | Griffin's Orientation Mode |
|---|---|---|---|---|
| | **Habitat Change** | **Life Stage** | **Cue** | |
| 9. | Wintering grounds to summer feeding grounds | immature | coordinate grid system | III |
| 10. | Feeding grounds to home river estuary | maturing adult | coordinate grid system | III |
| | | | odour/pheromone | I |
| 11. | Estuary to lake outlet | maturing adult | current | I |
| | | | odour/pheromone | I |
| 12. | Lake to mouth of spawning stream to spawning grounds | mature adult | exploratory | I |
| | | | celestial? | II |
| | | | magnetic? | II |
| | | | odour/pheromone | I |
| | | | current | I |

Chinook salmon generally spawn in large streams with fast flowing waters and gravel beds with large boulders. Depending on the stock, the fry may move to the estuary soon after emergence, or stay in the river for three months to a year (Figures 10A and 10B, C3) (Healey 1991). The fry that enter salt water soon after emergence form schools in the estuary. The ones that stay in the river move towards the river banks and set up feeding territories, much as the coho salmon fry do, but in somewhat deeper water away from the banks. The juvenile chinook salmon that overwinter in fresh water will migrate to the estuary during spring as smolts (Figure 10B, C3). Many coho and chinook salmon juveniles move north upon entering salt water (Figures 9, 10A and 10B, C4). Most stocks will stay in coastal areas; others, however, may move further northward as the pink and chum salmon juveniles do (Sandercock 1991; Healey 1991).

Sockeye salmon generally spawn in tributaries and outlet streams adjacent to lakes. The fry upon emergence from the gravel may spend from one to three years in lake nursery areas before migrating to sea as smolts. The movements of fry originating from populations spawning in tributaries and outlet streams differ. In both cases the fry emerge at night and initially operate as individuals, but tributary fry move actively downstream when leaving the spawning gravel beds, whereas outlet fry attempt to reach

| Age | Habitat change | Life cycle stage | | Timing |
|---|---|---|---|---|
| 1st year | 1. egg to gravel | alevin | | winter |
| | 2. gravel to estuary | fry | 0.0 | spring |
| | 3. estuary down inlet to sea | fingerling | 0.0 | summer |
| | 4. to ocean north along shore | juvenile | 0.0 | autumn |
| | 5. offshore to wintering grounds | immature | 0.0 | winter |
| 2nd year | 6. wintering grounds southwards to summer feeding grounds | immature | 0.1 | spring |
| | 7. feeding grounds to home river estuary | maturing | 0.1 | summer |
| | 8. home river estuary to spawning grounds | mature<br>adult | 0.1 | autumn |

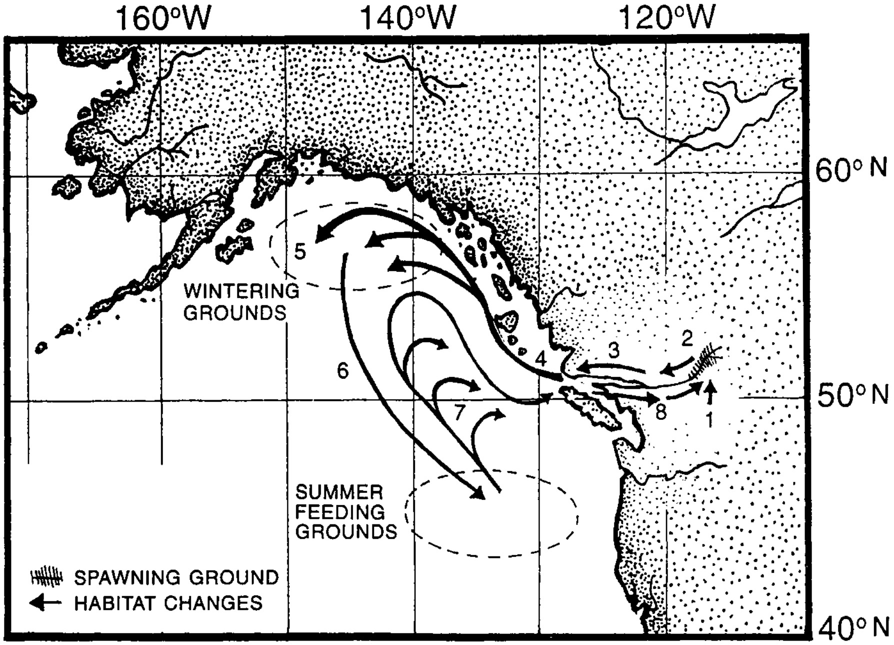


FIGURE 7. Habitat changes of pink salmon of the central British Columbia coast during their life cycle (from Groot 1982).

| Age | Habitat change | Life cycle stage | | Timing |
|---|---|---|---|---|
| 1st year | 1. egg to gravel | alevin | | winter |
| | 2. gravel to estuary | fry | 0.0 | spring |
| | 3. estuary down inlet to sea | fingerling | 0.0 | summer |
| | 4. to ocean north along shore | juvenile | 0.0 | autumn |
| | 5. offshore to wintering grounds | immature | 0.0 | winter |
| 2nd year | 6. wintering grounds to summer feeding grounds | immature | 0.1 | spring |
| | 7. summer feeding grounds to wintering grounds | immature | 0.1 | autumn |
| 3rd year | 8. wintering grounds southwards to summer feeding grounds | immature | 0.2 | spring |
| | 9. feeding grounds to estuary home river | maturing | 0.2 | summer |
| | 10. home river estuary to spawning grounds | mature adult | 0.2 | autumn |

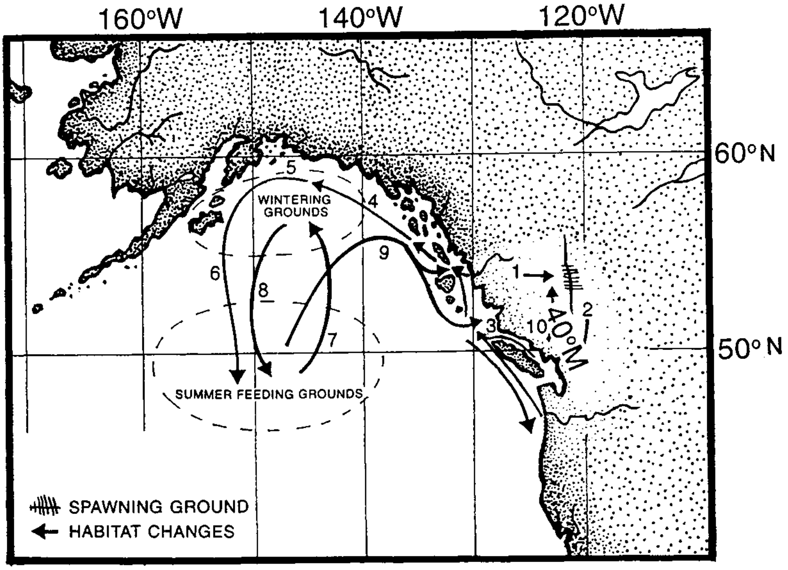


FIGURE 8. Habitat changes of 3-year-old chum salmon of the central British Columbia coast during their life cycle.

| Age | Habitat change | Life cycle stage | | Timing |
|---|---|---|---|---|
| 1st year | 1. egg to gravel | alevin | | winter |
| | 2. gravel to river shore or lake | fry | 0.0 | spring |
| | 3. up or down river to deeper parts | fingerling | 0.0 | summer/ autumn |
| 2nd year | 4. river or lake to estuary | smolt | 1.0 | spring |
| | 5. estuary north to wintering grounds | immature | 1.0 | summer/ autumn |
| 3rd year | 6. wintering grounds to summer feeding grounds | adult | 1.1 | spring |
| | 7. summer feeding grounds to home river | maturing adult | 1.1 | summer/ autumn |
| | 8. home river estuary to spawning grounds | mature adult | 1.1 | autumn/ winter |

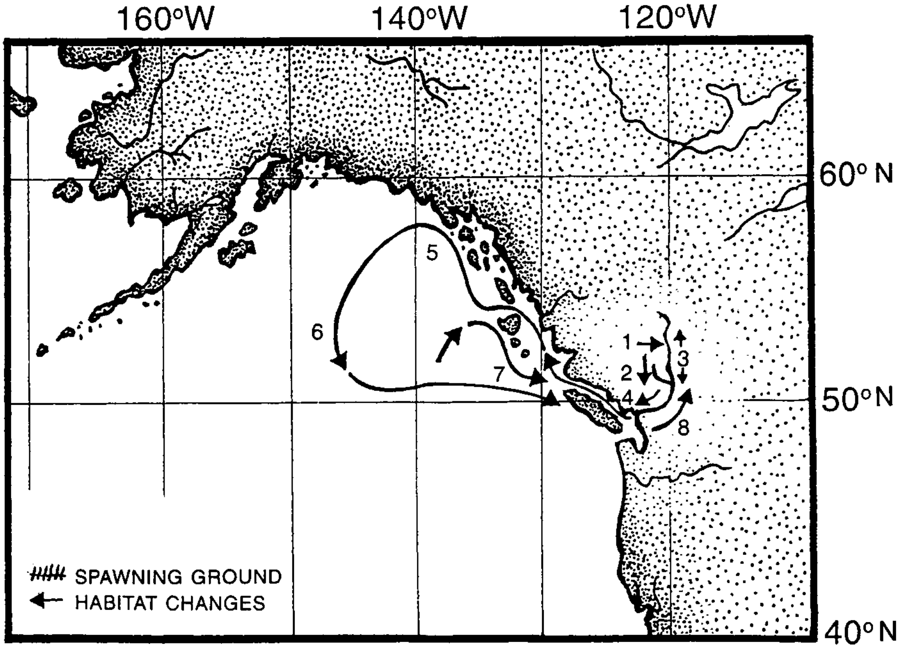


FIGURE 9. Habitat changes of 3-year-old coho salmon of the central British Columbia coast during their life cycle.

| Age | Habitat change | Life cycle stage | | Timing |
|---|---|---|---|---|
| 1st year | 1. egg to gravel | alevin | | winter |
| | 2. gravel to estuary | fry | 0.0 | spring |
| | 3. estuary to sea | fingerling | 0.0 | summer |
| | 4. offshore to wintering grounds | juvenile | 0.0 | autumn/ winter |
| 2nd year | 5. wintering grounds to summer feeding grounds | immature | 0.1 | spring |
| | 6. summer feeding grounds to wintering grounds | immature | 0.1 | autumn |
| 3rd year | 7. wintering grounds southwards to summer feeding grounds | immature | 0.2 | spring |
| | 8. feeding grounds to home river estuary | maturing | 0.2 | summer |
| | 9. home river estuary to spawning grounds | mature | 0.2 | autumn |

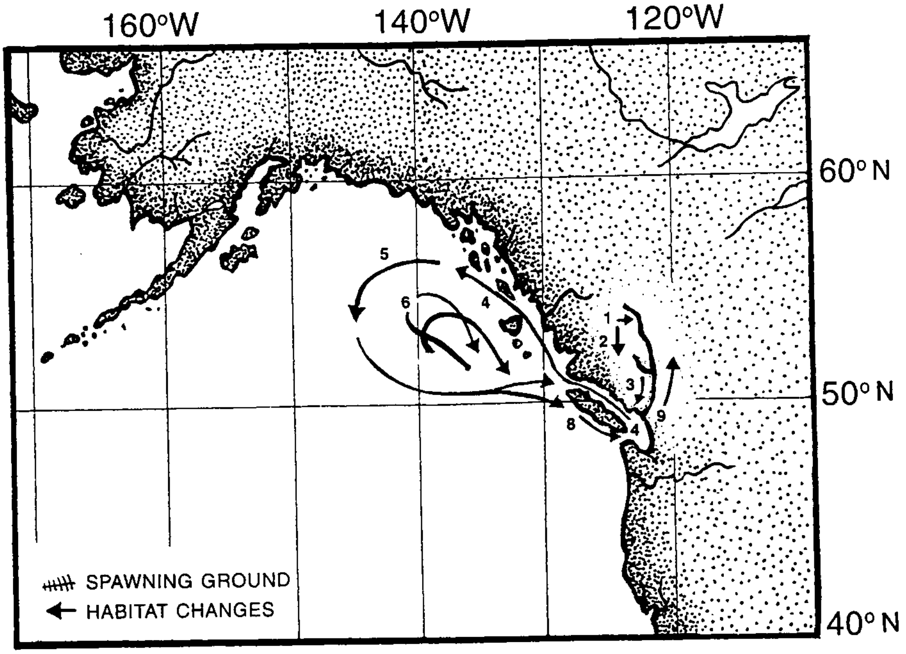


FIGURE 10A. Habitat changes of 3-year-old ocean-type chinook salmon of the central British Columbia coast during their life cycle.

| Age | Habitat Change | Life Cycle Stage | | Timing |
|---|---|---|---|---|
| 1st year | 1. egg to gravel | alevin | | winter |
| | 2. gravel to river | fry | 0.0 | spring |
| | 3. up or down river to deeper parts | fingerling | 0.0 | autumn |
| 2nd year | 4. river to estuary | smolt | 1.0 | spring |
| | 5. estuary to sea | juvenile | 1.0 | summer |
| | 6. offshore to wintering grounds | juvenile | 1.0 | autumn/ winter |
| 3rd year | 7. wintering grounds to summer feeding grounds | immature | 1.1 | spring |
| | 8. summer feeding grounds to wintering grounds | immature | 1.1 | autumn |
| 4th year | 9. wintering grounds to summer feeding grounds | immature | 1.2 | spring |
| | 10. feeding grounds to home river estuary | maturing | | summer |
| | 11. home river estuary to spawning grounds | mature | 1.2 | autumn |

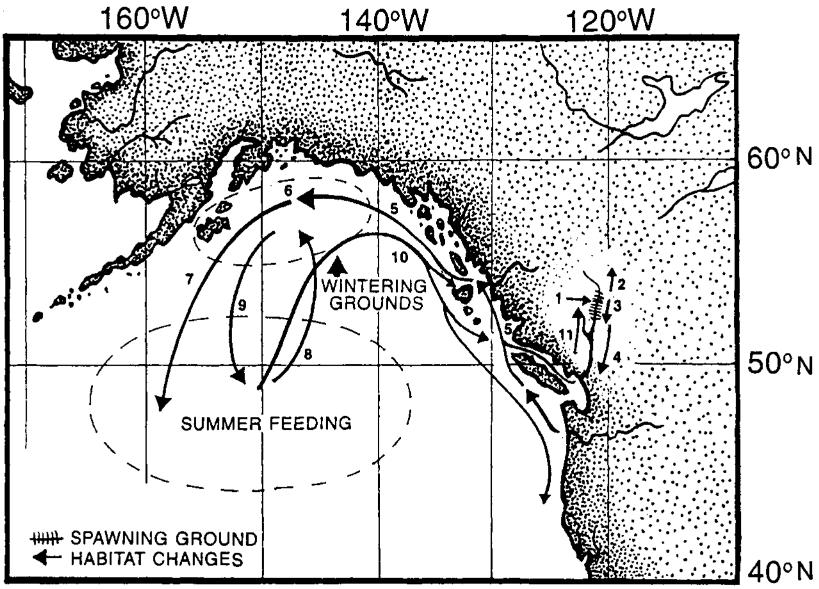


FIGURE 10B. Habitat changes of 4-year-old river- or stream-type chinook salmon of the central British Columbia coast during their life cycle.

| Age | Habitat change | Life cycle stage | | Timing |
|---|---|---|---|---|
| 1st year | 1. egg to gravel | alevin | | winter |
| | 2. gravel to lake | fry | 0.0 | spring |
| | 3. down lake | fingerling | 0.0 | summer/ autumn |
| 2nd year | 4. lake to estuary | smolt | 1.0 | spring |
| | 5. estuary north along shore | juvenile | 1.0 | summer |
| | 6. offshore to wintering grounds | immature | 1.0 | autumn |
| 3rd year | 7. wintering grounds to summer feeding grounds | immature | 1.1 | spring |
| | 8. summer feeding grounds to wintering grounds | immature | 1.1 | autumn |
| 4th year | 9. wintering grounds southwards to summer feeding grounds | immature | 1.2 | spring |
| | 10. feeding grounds to home river estuary | maturing | | summer |
| | 11. home river estuary to nursery lake | mature adult | 1.2 | autumn |
| | 12. lake to river spawning grounds | mature adult | 1.2 | autumn |

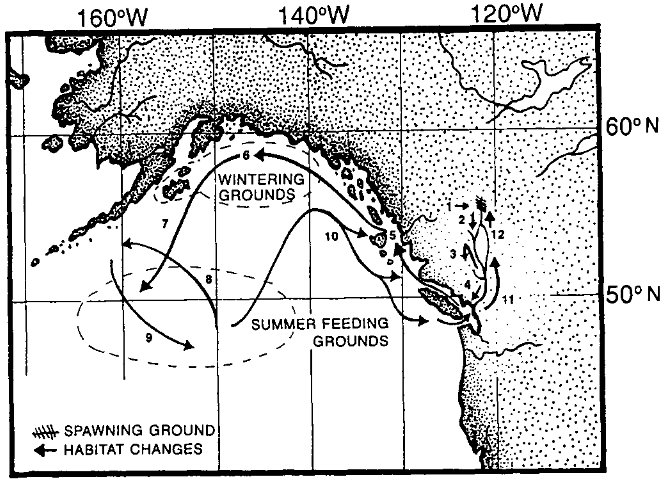


FIGURE 11. Habitat changes of 4-year-old sockeye salmon of the central British Columbia coast during their life cycle.

the river banks as soon as possible and then start to swim upstream to the lake (Figure 11, C2) (McCart 1967; Brannon 1972). In most instances, the journey downstream from the tributary spawning grounds to the lake is completed in one night. In the lake the fish abandon their solitary habits, become positively phototactic, form schools, and swim and feed in brightly lit areas along the lake shorelines. They remain along the shore of the lake for a few days and then disappear into deeper waters to take up a pelagic existence (Figure 11, C3) (McDonald 1969).

In the open waters of the lake, sockeye salmon juveniles perform diel vertical movements, rising to surface waters at dusk, remaining there during the night, and descending at dawn to a daytime level of 16 m or more (McDonald 1969; Levy 1987). The pelagic existence persists until the following spring when the juveniles change into smolts and migrate out of the lake down river to salt water (Figure 11, C4) (Johnson and Groot 1963). After reaching salt water young sockeye are not seen in inshore waters for long. They move offshore rather quickly and migrate northward with juvenile pink, chum, coho and chinook salmon in a narrow band along the coast until they reach the northern Gulf of Alaska to overwinter in northern waters (Figure 11, C4).

During ocean residence Pacific salmon move relative to the annual temperature cycle in the subarctic North Pacific Ocean. They are in waters colder than 7°C in winter, 10.5°C in spring, and 15°C in summer (Welch et al. 1995). They thus move south in spring and summer (Figures 7 and 8, C5; Figure 11, C6) and north in autumn and winter (Figures 7 and 8, C6; Figure 11, C7) continuously changing habitats.

Pink and coho salmon spend only one winter at sea. The maturing adults move south in spring and then north again later in summer before they migrate towards the outlets of their home streams (Figures 7 and 9, C7) (Heard 1991; Sandercock 1991). Sockeye, chum and chinook generally stay in the ocean for two or more winters before embarking on their homeward migration (Figures 8, 10A and 10B, C7; Figure 11, C8) (Burgner 1991; Healey 1991; Salo 1991). Depending on the species and stock, Pacific salmon may hold for up to several weeks before they migrate upriver to the spawning grounds (Figures 7, 8, 9, 10A and 10B, C8; Figure 11, C9). Sockeye salmon generally spend some time in their nursery lake before moving on to tributary or outlet spawning areas. The other species swim upriver; pink, chum and coho salmon generally for short distances, and chinook and some chum salmon stocks for distances up to 2,000-3,000 km in some large rivers, such as the Yukon and Mackenzie rivers (Groot and Margolis 1991).

### *Preparatory Phase*

In the preparatory phase the fish undergo biological modifications that result in a state of migration readiness or migratory disposition. These are most obvious in cases of a habitat shift from fresh water to salt water or the reverse. The biological changes accompanying the parr-smolt transformation have attracted much attention from researchers during the last 40 yr (Hoar 1976; Wedemeyer et al. 1980; Clarke and Hirano 1995) and are discussed in detail in Chapter 8. The salient points for the preparatory phase of migration of salmonids have been summarized by Groot (1982) as follows:

(1) There is a strong endogenous component involved in triggering the diel and seasonal morphological, physiological, and behavioural changes during the parr-smolt transformation.

(2) This endogenous rhythm only becomes functional when the fish reach a certain size (physiological age), especially in coho and Atlantic salmon, and steelhead trout.

(3) Photoperiod entrains the endogenous rhythm and thus controls the timing of the onset of smolting.

(4) Temperature influences the onset of smolting through growth and regulates the magnitude and duration of the smolting process.

(5) Hormones act as chemical links between organism and the environment.

(6) The coordination of smolting processes leading towards migration readiness is attained in different ways for separate stocks as a result of adaptations to environmental factors operating in the habitat.

### *Seasonal Cycles of Migration*

Migratory readiness in juvenile salmonids is transitory in nature. Smolts retained in fresh water past a certain critical period for migration readapt their physiology to the freshwater environment (Baggerman 1960*b*; McInerney 1964; Hoar 1976). The following spring the parr-smolt transformation is reactivated. Under natural conditions this re-occurring and waning of the smolting process, without culminating in actual migration, may occur a number of times in juvenile coho, sockeye, and Atlantic salmon and steelhead that leave fresh water for the ocean after one or more years. Coho and sockeye salmon fry also show a brief period of saltwater preference immediately after emergence, although they generally stay in fresh water until after smolt transformation (Baggerman 1960*a*, 1960*b*; McInerney 1964). Hoar (1965) postulated that a temporal pattern of changing salinity tolerance exists in salmonids, from complete survival in high salinity during summer and autumn to a very low level of resistance in winter and early spring. Hoar assumed that this is a basic pattern of physiological changes for salmonids of all age classes and proposed the following general theme for salmonid migrations.

Firstly, salmonids are endowed with a physiology that favours life in fresh water during winter and spring and a life in a marine habitat during summer and autumn, at all stages of development (Figure 12). Secondly, this seasonal basic rhythm is

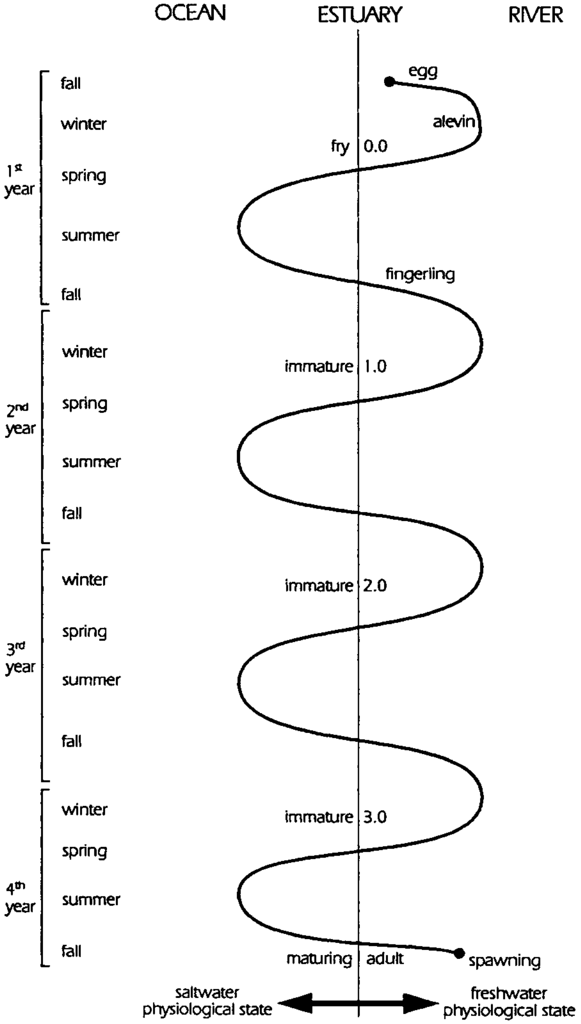


FIGURE 12. The hypothetical pattern of changes in salmonids between a freshwater physiological state during autumn and winter and a saltwater physiological state during spring and summer, resulting in regularly occurring habitat changes between ocean and river environments. Adapted from Hoar (1965).

endogenous and is adjusted by photoperiod. Thirdly, during evolution of the salmonids the basic rhythm of this cycle has been modified in various ways by lengthening, shortening, or otherwise altering the different components to produce a spectrum of migratory types. Following Hoar's (1965) example, the migratory patterns of pink, chum, coho, chinook and sockeye salmon are represented as shifts between freshwater and saltwater physiological states in Figures 13 to 17 to illustrate the modifications in these species that have resulted in the different life histories.

Although there are still a great number of unanswered questions with respect to the anadromous migratory behaviour of salmonids, Hoar's (1965) working hypothesis of a seasonally changing physiology in the salmonids and the possible modifications that might have occurred during evolution, resulting in a spectrum of migratory types, is very attractive. It certainly identifies a major theme around which a great number of variations and adaptations could have developed in relation to environmental conditions.

### *Migratory Phase*

Migration in salmonids, especially in Pacific salmon, is often a dramatic event. One day there are no fish showing and then suddenly, the whole population is on the move. Because of the funnelling effect of estuaries, rivers, and tributaries, and the large number of fish involved, the migrations of juvenile and adult salmonids are often very visible dramatic events. These major phenomena of salmonid migration happen every year along the coasts of North America, Europe, and northern Asia, within specific time windows. Even the casual observer must be struck by the well-timed and well-directed character of these movements. Fish migration has three components: speed, depth, and direction of movement. All three components show changes with time of day, time of season, and life cycle stage, and are affected by certain environmental conditions.

In sockeye salmon, emerging fry from tributary streams move downstream at night, whereas subsequent migration into the lake environment or movements along the lake shore, occur during daylight hours (McCart 1967). When the juveniles move to deeper waters to take up a pelagic life, they change to a dusk-dawn activity pattern (McDonald 1969). Smolt migration also peaks at dusk and dawn (Groot 1965; Hartman et al. 1967), whereas the adults returning to spawn may move in day and night, sometimes with a slightly higher level of activity during the day (Madison et al. 1972; Ellis 1962; C. Groot, unpubl. data).

The speed of migration changes with time of day and net displacement increases as the season progresses (Johnson and Groot 1963; Madison et al. 1972; Groot 1972; Hartt and Dell 1986). Johnson and Groot (1963) reported that sockeye salmon smolts in Babine Lake, British Columbia, increased the distance covered per day with time of season. This increase could not be accounted for by increasing temperature and flow rates alone and was assumed to result from a rise in migration drive. Hartt (1966) found that maturing adult sockeye returning to Bristol Bay, Alaska, from the ocean feeding grounds increased their rate of travel significantly with time from May to July.

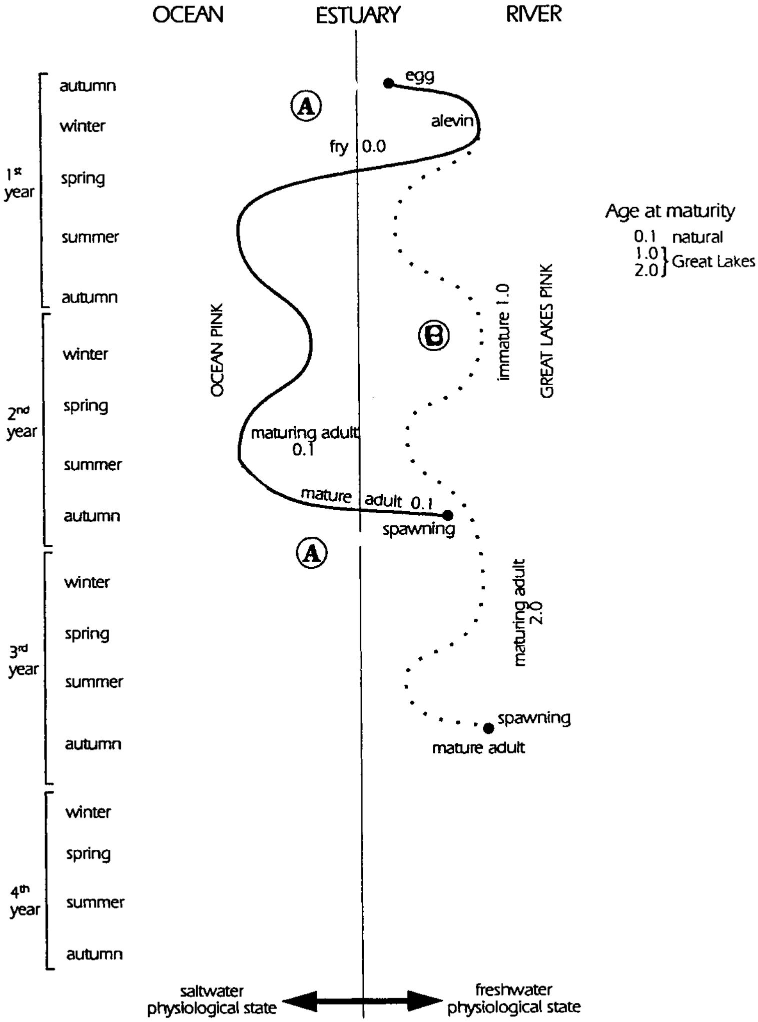


FIGURE 13. Seasonal changes between freshwater and saltwater physiological states for pink salmon. (A) 2-yr-old pink salmon in coastal areas; and (B) 3-yr-olds in the Great Lakes. Adapted from Groot (1982).

Salmonids generally tend to rise to the surface of river, lake, and ocean during migration (Manzer 1964; Groot 1965, 1972; McCart 1967). Some authors have speculated that this may be related to celestial orientation (Johnson and Groot 1963; Neave 1964; Groot 1965, 1972; McCart 1967). Brett (1971, 1995) and Levy (1987) argued that the maintenance of greater depth of sockeye in lakes and possibly in the ocean during the

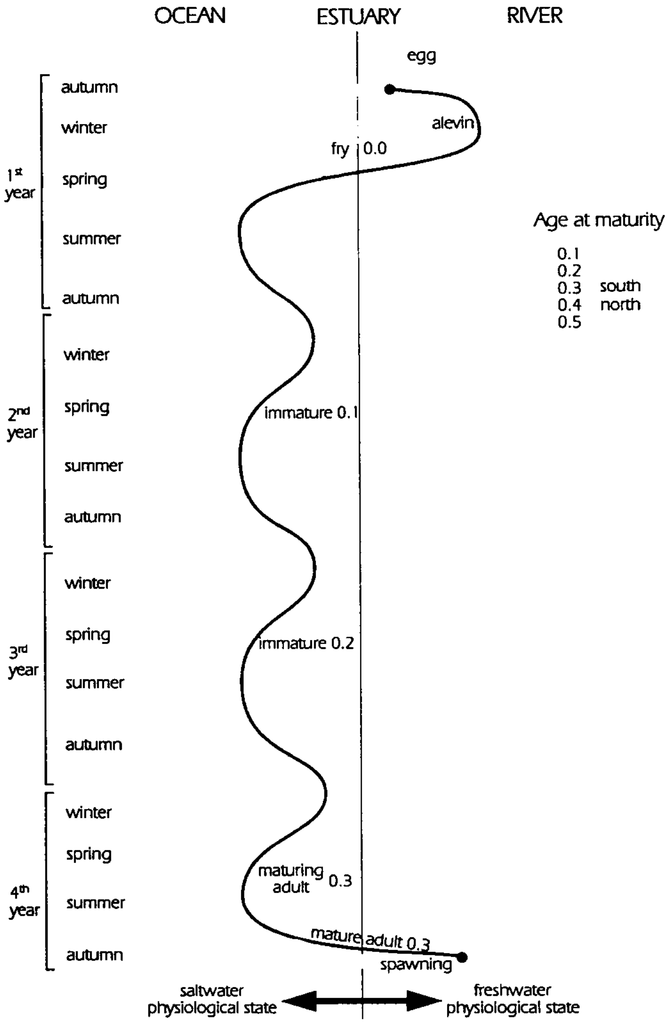


FIGURE 14. Seasonal changes between freshwater and saltwater physiological states for 4-yr-old chum salmon.

day may have metabolic advantages by reducing rates of energy conversion. For increased levels of activity the fish must rise to the surface to move into waters of higher temperature. In this case, migration can be considered as a number of hops during which fish rise to the surface during peak times of activity, either by day or night, and return to greater depths into areas of colder temperature during periods of low activity.

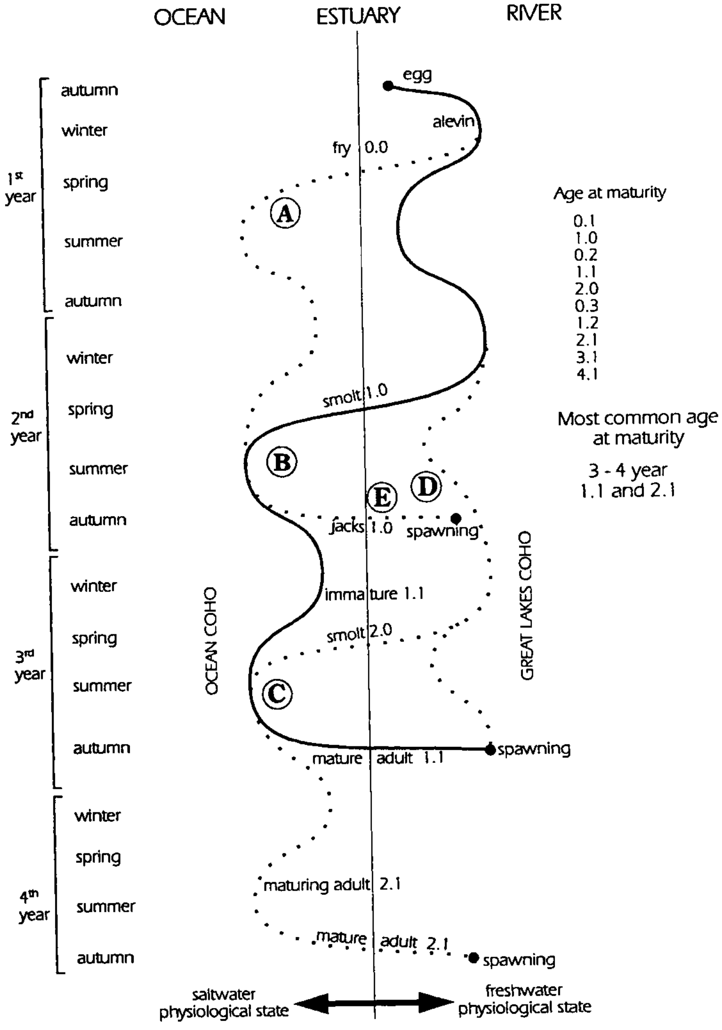


FIGURE 15. Seasonal changes between freshwater and saltwater physiological states for coho salmon. (A) entering saltwater as fry; (B) 3-yr-old coho salmon after 1 year in freshwater; (C) 4-yr-old coho salmon after 2 yr in freshwater; (D) total fresh water existence for 3-yr-old coho salmon; and (E) 2-yr-old coho salmon jack.

The ability of salmonids to orient in space and time is impressive, as is illustrated by the many long distance movements they perform during their life cycle. The extraordinary capabilities of salmon to return to the stream of origin, often to the exact place of birth in a river system after several years and many thousands of kilometres of

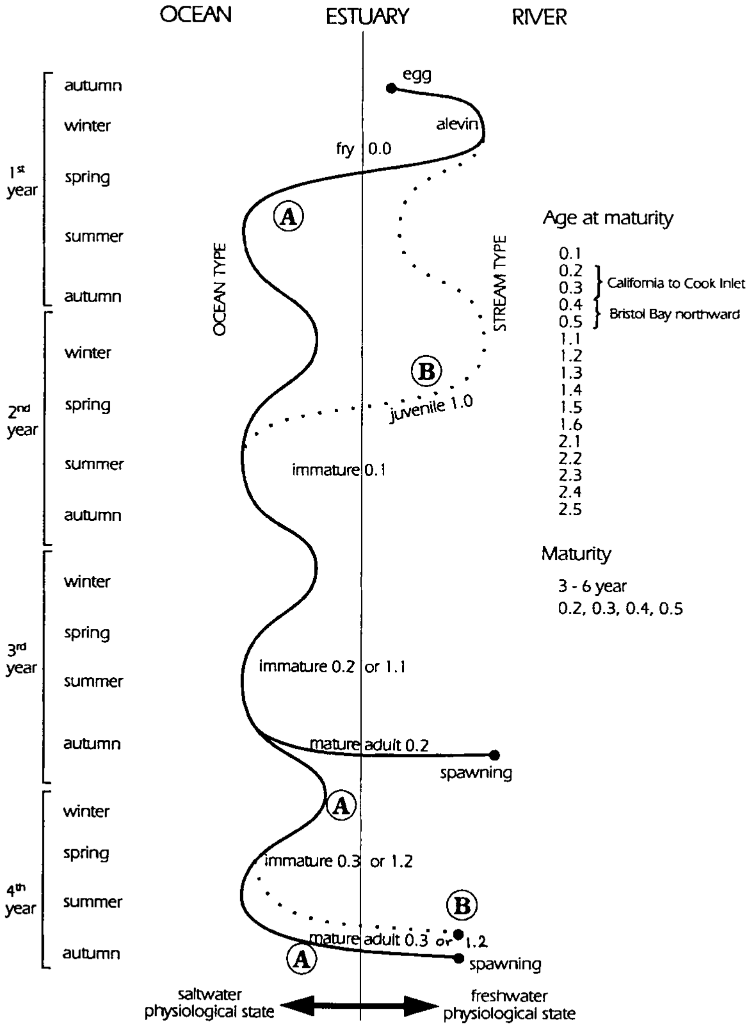


FIGURE 16. Seasonal changes between freshwater and saltwater physiological states for chinook salmon. (A) 3- and 4-yr-old ocean-type chinook salmon; and (B) 4-yr-old stream-type chinook salmon.

travelling in river, lake, and ocean, suggest that salmonids have well developed direction finding systems.

Griffin (1955) defined three levels of orientation ability in animals. The first level, Type 1, is defined as a simple reliance on visual and other environmental marks within

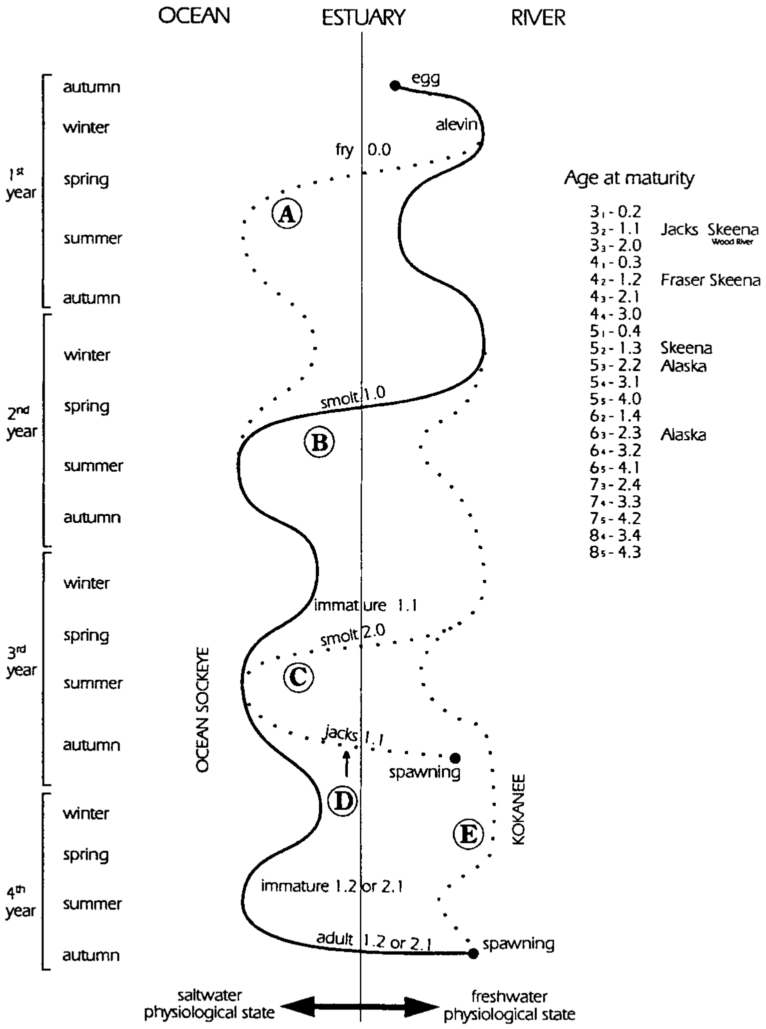


FIGURE 17. Seasonal changes between freshwater and saltwater physiological states for sockeye salmon. (A) entering salt water as fry; (B) for a 4-yr-old sockeye salmon after 1 year; (C) 2-yr smolt stage; (D) for a 3-yr-old sockeye salmon jack; and (E) for a total freshwater existence of a 4-yr-old kokanee. Adapted from Groot (1982).

familiar territory and as exploration or undirected searching in unfamiliar territory. Type 2, generally called compass or one-direction orientation, is the ability to move in a particular compass direction without reference to environmental marks. Type 3, which is called goal- or bico-ordinate orientation, or true navigation, refers to the ability of an animal to orient towards a specific goal from a variety of unfamiliar areas. The significant difference between Type 2 and 3 is that compass orientation is generally performed by juveniles or young of the year that are migrating away from the breeding grounds for the first time. This orientation is considered innate. Goal orientation or navigation apparently can only be performed by older animals that have previous experience of the goal, i.e., wintering, feeding, or breeding grounds (Perdeck 1958). Although goal finding includes components of compass orientation, it also involves a learning process about the goal.

The orientation abilities of salmonids, which have been experimentally identified, and those for which there is strong circumstantial evidence are briefly reviewed as follows. Starting with the habitat changes of young salmonids in the incubation environment, Bams (1969) concluded that alevins respond in a negative and fry in a positive geotactic way to gravity during, respectively, the "downward" and "upward" phases of movement in the gravel. When the passage upwards through the gravel during emergence is blocked, the fry change their behaviour and respond positively to current (positive rheotaxis), by swimming horizontally until they encounter a better place to surface.

Pacific salmon fry leave their gravel beds at night. A negative response to light (negative phototaxis) keeps them in the gravel until dark (Bams 1969). After emergence at night, they actively swim downstream with the current (negative rheotaxis) (McCart 1967; Hartman et al. 1967; Brannon 1972). With the appearance of dawn, this migration stops and the fry dive to the bottom to hide in the gravel (negative phototaxis) (Hoar 1958; McDonald 1960). This behaviour changes within a few days, especially if open waters of the estuary or lake have been reached, from a nocturnal to a diurnal activity pattern and from swimming as individuals to moving in schools.

Brannon (1972) determined that fry from different sockeye populations could discriminate between odourant qualities of lake and non-lake waters. Bodznick (1978*a*) followed up on these observations and showed that two separate factors are involved. There is "an apparent innate preference for lake water as compared to non-lake water and for the water source that was familiar following recent experience over foreign waters". Sockeye salmon fry tested in non-lake waters were more negatively phototactic than those tested in waters of lake origin (Bodznick 1978*a*). This may be of added importance for tributary spawning populations to avoid unnecessary upstream migrations. Further experiments by Bodznick (1978*b*) indicated an acute sensitivity of sockeye fry to calcium ions and he suggested that this ion might be one of the important odourant components in lake water the fry are responding to.

Pacific and Atlantic salmon juveniles are capable of compass orientation (Type 2) (Johnson and Groot 1963; Groot 1965; Healey 1967; Brannon 1972; Quinn 1980; Brannon et al. 1981; Quinn and Brannon 1982). When tested in experimental arenas they show directional choices parallel to the migration directions they follow in the ocean. Both celestial (sun) and magnetic cues are used for direction finding, but the relationship between the guidance systems is complex (Groot 1965; Quinn 1980, 1982; Quinn and Brannon 1982).

Juvenile salmonids also respond to e-vector changes of plane polarized light under both natural and experimental conditions (Groot 1965; Dill 1971; Hawryshyn et al. 1990). The polarized light pattern of the sky is complex and is dependent on the position of the sun. The ability to determine the e-vector of plane polarized light may aid young salmonids in determining the position of the sun when the sun is not directly visible. Hawryshyn (1992) has further shown that juvenile coho salmon and rainbow trout are also capable of perceiving ultraviolet light and has demonstrated that they respond to shifts in the plane of polarization of such light. This ability could aid the fish to determine the position of the sun under overcast sky conditions.

The directional preferences of both fry and smolts during migration appear genetically fixed for each stock. Even in one lake system, different sockeye salmon smolt stocks can have different directional preferences that are appropriate for each stock to reach the outlet of the lake (Johnson and Groot 1963; Groot 1965). Smolts entering the river swim actively with the current, suggesting negative rheotaxis. Not much is known about migratory patterns in estuaries. Chinook and chum salmon juveniles usually spend some time in brackish water areas, but sockeye salmon and steelhead smolts seem to move right through to continue their migration (Manzer 1956). McInerney (1964) showed that fry of pink salmon and of most other Pacific salmon show a gradually increasing saltwater preference in spring. This changing pattern is reasonably well correlated with the salinity differences naturally migrating salmon juveniles would encounter in long inlets. McInerney (1964) therefore hypothesized that a changing salinity preference might be used by juvenile salmon as a means of direction-finding through inlets and possibly large estuaries.

Although there is no experimental evidence of the orientation capabilities of immatures and maturing adults in the ocean, it is reasonable to assume that those mechanisms found for fry and smolts, especially the celestial and magnetic orientation abilities, are also part of the direction finding repertoire of ocean life stages. Royce et al. (1968) speculated that salmon might also use natural electric phenomena in the ocean for orientation during migration. The vast wind-driven ocean streams moving through the earth's magnetic field generate large-scale electric induction fields. Because these fields are related to the velocity of the current and are polarized with respect to the flow, they can theoretically inform electrosensitive animals of upstream and downstream directions and speed of the ocean current (Kalmijn 1974). Movement of fish, relative to the magnetic field, also induces a electric field, and is a potential source of compass orientation. Juvenile salmonids along both the North American and Asiatic coasts migrate in narrow bands along the continental coasts and move with the current upon leaving their home stream (French et al. 1976; Takagi et al. 1981; Hartt and Dell 1986; Groot and Cooke 1987). Because these migrations occur in areas of strong currents, which will induce electric voltage gradients, Royce et al. (1968) hypothesized that currents could serve as orientation cues during the long-distance journeys. Such orientation abilities have been shown for sharks and rays that have electric-sensing organs. McCleave et al. (1971) and Rommel and McCleave (1973) obtained some experimental evidence indicating that Atlantic salmon are sensitive to electric fields of the order generated by ocean currents. However, there are still various theoretical and experimental problems to be solved before the possibility of electro-orientation in salmonids in the ocean can be seriously considered (Kalmijn 1974).

There is no direct experimental evidence that salmonids are capable of Type 3 orientation or true navigation but the circumstantial evidence is strong. For example, sockeye salmon from Bristol Bay, Alaska, return to their home area from a spread of greater than 3,700 km in the North Pacific Ocean in an east-west direction south of the Alaskan Peninsula and arrive at Bristol Bay within a 9- to 22-d period (Burgner 1991). Some fish have to migrate from as far as 2,220 km away, either directly or around the Alaskan Peninsula and are able to do this at average speeds ranging from 44.5 to over 60 km per day (Hartt 1966; Royce et al. 1968; French et al. 1976). Because the optimum sustained travelling speed of migrating sockeye salmon is between 1.9 and 2.4 km/hr (Madison et al. 1972; Groot and Quinn 1987; Quinn et al. 1989), these fish must migrate day and night at their optimal sustained swimming speed in a very directed way irrespective of whether the starting points were east or west from Bristol Bay. The accuracy of homing appears to be independent of whether the salmon could take a direct route through the passes of the Aleutian Islands, or had to circumnavigate the Alaskan Peninsula (Groot 1982).

Information on distributions of Pacific salmon in the North Pacific Ocean collected by Canada, Japan, and the U.S. as members of the International North Pacific Fisheries Commission, indicates that the Bristol Bay sockeye salmon are not an isolated case, but that many salmon stocks perform similar remarkable feats of directed movements and accurate timing during their homing migrations. Such observations strongly suggest that Pacific salmon are capable of true navigation (Type 3 orientation ability) in finding their ancestral spawning rivers from the immense stretches of Pacific Ocean over which they are distributed during their ocean life. Neave (1964) stated that, "It is difficult to avoid the conclusion that throughout the period of ocean life some awareness of position in relation to the place of origin is maintained."

Little is known about the mechanisms involved in the homing process. In 1953 Kramer proposed the map-compass concept as a two-step process. As part of the compass component salmonids could be using celestial and the earth's magnetic cues to maintain a compass direction toward a goal, but we are no closer in understanding the "map" component by which they determine their geographic position in relation to the goal.

When salmon reach their home river to migrate upstream to the spawning grounds they respond to odours of the home stream to which they presumably imprinted during the juvenile stages (Hasler 1971; Hasler and Scholz 1983). The odours appear to act as releasing stimuli, with the current acting as the directing agent (Brett and Groot 1963). Salmon have been observed to choose the "wrong" arm of a river at a cross point and then backtrack after a few kilometres to finally enter the "right" arm (Foerster 1968). It is also possible that pheromones from conspecifics play a role during the upstream migration (Nordeng 1977). Groot et al. (1986) have shown that sockeye salmon migrating up one river to two adjacent lake systems are capable of distinguishing between the body odours of their own and the neighbouring stock.

Migration upstream of Pacific salmon in big rivers such as the Fraser River, British Columbia, takes place close to shore and close to the surface (T. Mulligan, Pacific Biological Station, Nanaimo, B.C., Canada, pers. comm.). In smaller rivers, Ellis (1962) noted that movement of sockeye salmon through slow water normally occurs along the line of the deepest channel wherever it is well defined. Positive rheotaxis and use of landmarks is suggested in these cases. Findings of several researchers indicate that salmon at this stage of their migration strongly respond to specific chemical agents of home-stream water and actively swim against the current (Hasler 1971; Hasler and Scholz 1983; Brannon et al. 1984).

Thus, during the life cycle of sockeye salmon, and presumably of all Pacific salmon and other salmonids as well, a variety of cues can be used for direction finding during movements and migrations. In those situations where more than one mechanism has been identified for orientation, available information suggests that there are different weightings. Bams (1969) noted that during the upward moving phase of fry prior to emergence from the gravel, geotaxis takes precedence over rheotaxis. Groot (1965), Quinn (1980, 1982), and Quinn and Brannon (1982) found that sun-compass orientation is used preferably by sockeye fry and smolts and that they only switch to magnetic orientation when celestial cues are not available for direction finding. Brannon (1972), Brannon et al. (1981), and Quinn (1980, 1981, 1982) have shown that there are population differences in the orientation behaviour of sockeye salmon fry. The directional choices of fry from three different populations (Weaver Creek and Cedar and Chilko rivers, British Columbia) under experimental conditions were adaptive with respect to the types of migrations these populations have to perform to reach their nursery lakes. They also reported that the relationships between orientation mechanisms, such as rheotaxis, and celestial and magnetic orientation, can change at different life history stages. For example, the fry from Weaver Creek, which have to perform a rather complicated migration to reach their nursery lake, have to swim downstream first in Weaver Creek and Morris Slough and then upstream through Harrison River into Harrison Lake. These fry did not respond to celestial and magnetic cues as strongly as the Cedar and Chilko River fry did.

## SPAWNING BEHAVIOUR

Spawning behaviour among the salmonids shows a general pattern that is very similar for most of the species (Fabricius and Gustafson 1954; Jones and Ball 1954; Jones 1959; Hartman 1969; Schroder 1973, 1981; Tautz and Groot 1975; Leggett 1980). Arctic and lake charr show significant differences from this general pattern (discussed later).

Basically, the spawning behaviour of salmonids consists of a combination of nestbuilding activities by the female and courtship display by the male, leading to deposition of fertilized eggs in the nest. The sequence of activities are illustrated in Figure 18 and includes nest site selection (phase 1), followed by nest construction and courtship (phases 2 and 3), leading to oviposition and fertilization (phase 4), closing of the nest (phase 5), and defence of the redd with the buried egg pockets (phase 6). Finally both female and male become covered with fungus, lose locomotory control and die (phase 7). The female is the dominant partner in this process, in the sense that she selects the nest site, digs the nest, regulates the courtship activities towards oviposition by the progress and shape of the developing nest, and then protects the redd when all the eggs have been laid. Females generally stay in one area once a choice of a suitable nest building site has been made. Males, on the other hand, are transitory and have a tendency to move from one spawning female to another after each spawning act.

### *Nest Site Selection*

Nest site selection by the female is influenced by water depth, velocity, upwelling, accelerating flows, gravel composition, presence of cover, and whether or not the site is occupied by other females (Fabricius and Gustafson 1954; Schroder 1973, 1981; Tautz and Groot 1975; Duker 1977; Leggett 1980). Many salmonids prefer to spawn immediately above turbulent areas, or where there is upwelling through the gravel.

The primary tactic of the female is to search for an unoccupied space without fighting. Potential nest building sites are explored by "nosing" or "searching" behaviour with the head pointed toward the gravel substrate (often in contact with it) and the body elevated at a 7-15° angle (Figure 18, Phase 1). With pectoral, ventral, and caudal fins fully extended and often touching the gravel, and with head undulating from side to side, she slowly moves over the gravel upstream (Figure 19A). After a "nosing" bout she usually turns back and digs into the substrate just passed over. Prior to final nest selection the female may nose and dig (exploratory digging) over a fairly large area.

Females attack each other when establishing a territory and digging a nest. However, prolonged and intense confrontations rarely occur. The interactions that do transpire tend to distribute the females uniformly over the breeding ground. When competition for space becomes acute, as often occurs in sockeye, pink and chum salmon, nest sites form a tight mosaic. Under such conditions a proportion of ripe females wait

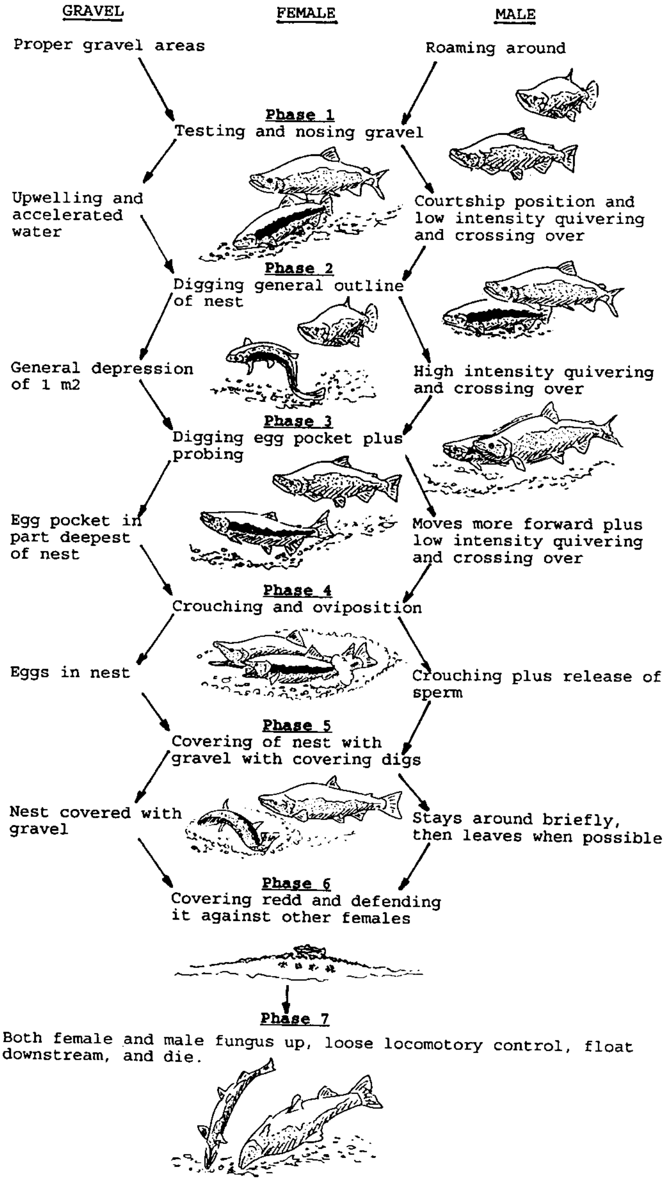
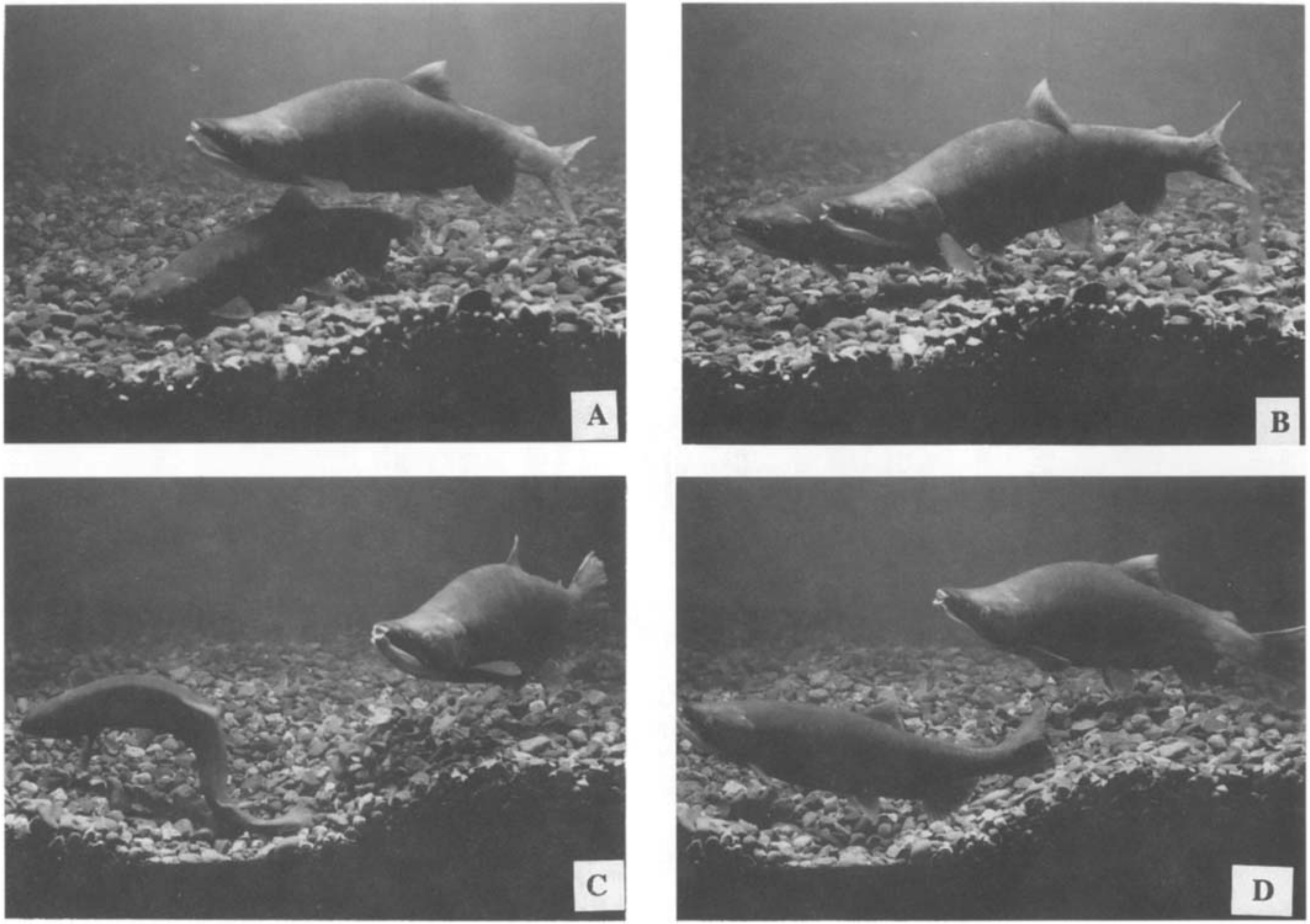

GRAVEL
FEMALE
MALE
Proper gravel areas
Roaming around
Phase 1
Testing and nosing gravel
Upwelling and accelerated water
Courtship position and low intensity quivering and crossing over
Phase 2
Digging general outline of nest
General depression of 1 m2
High intensity quivering and crossing over
Phase 3
Digging egg pocket plus probing
Egg pocket in part deepest of nest
Moves more forward plus low intensity quivering and crossing over
Phase 4
Crouching and oviposition
Eggs in nest
Crouching plus release of sperm
Phase 5
Covering of nest with gravel with covering digs
Nest covered with gravel
Stays around briefly, then leaves when possible
Phase 6
Covering redd and defending it against other females
Phase 7
Both female and male fungus up, loose locomotory control, float downstream, and die.

FIGURE 18. Sequence of spawning behaviour phases showing the interaction between female, gravel substrate, and male.

in groups in deeper parts of the stream until gravel areas become available. The waiting females make frequent cruises over the spawning grounds to check for areas that have


A
B
C
D

become vacant because their occupants died, or to test whether spawned out females that are weak can be evicted. There is a certain time limit within which spawning must occur, which is probably about 2-3 wk for Pacific salmon. Females that are delayed too long die without spawning.

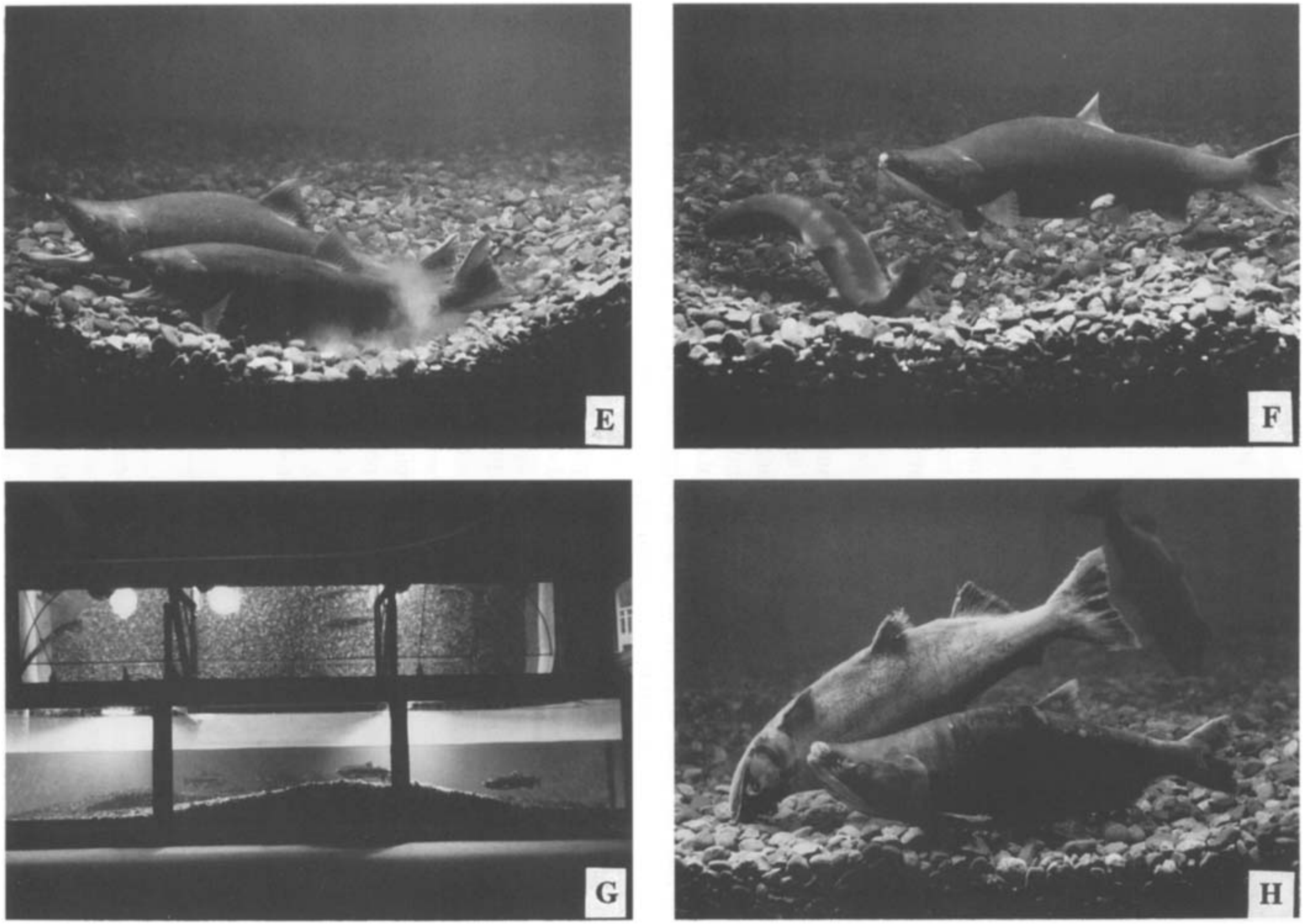
### *Nest Construction*

In digging a nest the female turns on her left or right side and performs a series (four to six) of rapid body flexures, slapping her tail on the gravel substrate (Figure 19C). During each dig the pectoral fins are held perpendicular to the body surface and appear to function as brakes. After each dig the female normally turns around and circles back to the rear part of the nest site. Besides turning and circling, she also "weaves" by swimming in tight circles or in a figure-eight pattern over the nest (Tautz and Groot 1975).

Digging activity initially occurs by moving radially from the back of the nest upstream in different directions. This creates an excavation of about 1 m in diameter and 20-40 cm deep with a porous layer of gravel at the bottom (Figure 18, Phase 2). As nest construction progresses digging is more and more concentrated at the central part of the nest (Figure 18, Phase 3). The tendency for the female to turn and circle after each dig commonly decreases when the nest nears completion. Instead, she often lets the current push her backwards into the nest depression after a dig.

During the latter stages of nest preparation, when the nest has developed a centralized depression, the female will stand over the deepest portion of the bed and slowly lower her midbody with the tail bend upwards and the anal fin extended. This is called a "probe" (Figure 18, Phase 4; Figures 19D, 20A and 20B) (Tautz and Groot 1975). When the anal fin touches the gravel or slips between the larger stones at the bottom, she bounces back to her original horizontal position. This activity may be followed by a weave, a dig, or another probe. Probing rapidly increases in intensity about one hour before spawning. As the nest becomes deeper, the angle of the body during a probe becomes more and more pronounced and it appears that it is used by the female to monitor the structure of the nest. Generally, probing decreases about 30 min before spawning commences. When angle of the probe reaches approximately 20°, the nest is complete (Tautz and Groot 1975).

FIGURE 19 (*opposite page*). Spawning behaviour of sockeye salmon observed in a large aquarium. (A) Female in the process of selecting a nest site by nosing and testing gravel with outstretched fins. The male is waiting in the courting position. (B) Courting pair over nest site with male moving forward from courting position to quiver. (C) Female digging a nest. The eggs will be laid in a small pocket where her tail fin is. (D) Female moving into nest to probe by pushing her anal fin into the gravel at bottom of nest.


E
F
G
H

Fabricius and Gustafson (1954) described two different types of digging movements in Arctic charr. At first, the female performs rapid and powerful swimming movements while remaining upright on the spot over the nest site. This "sweeping" behaviour produces a jet of water backwards that sweeps sand and other loose material away. The second kind of digging by Arctic charr is of the common salmonid type. Lake charr spawn primarily in lakes along exposed shorelines or on mid-lake shoals. A similar sweeping behaviour to clean spawning shoals is apparently performed by the females of these fish (DeRoche 1969).

### *Male Courtship*

As soon as a female has selected a nest site and starts to nose, turn, and weave, she is joined by a male (Figure 19A). Males pay much more attention to active females than to wandering, motionless, and spent ones (Schroder 1981). After joining a digging female the male often pushes his nose under her vent, as if to check her reproductive condition. Following this action, the male may move on or remain and take up the courting position just above and behind the female, with his head near or behind her dorsal fin (Figures 19A, 20A and 20B).

The principal courtship behaviours of the male consist of "quivering", a darting movement towards the female accompanied by short and rapid shaking of the head and body (Figure 19B), and "crossing-over", a side to side movement over the caudal peduncle region of the female (Tautz and Groot 1975). The first courting movement a male performs after locating a female is often a quiver. Both crossing-over and quivering increase in frequency as the nest nears completion. This coincides with a decrease in weaving and turning and an increase in probing by the female. Most notable is the association between quivering and probing.

The general sequence of behaviour during the later stages of courtship display is usually as follows; after completing a dig (Figure 19C) the female drifts backwards so that her vent is over the pocket, where the eggs will be deposited, and initiates a probe (Figure 19D). The male then approaches her, while quivering, from his position behind and to one side of her (Figure 19B). At this point, the female has usually completed the

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FIGURE 19 cont'd. (*opposite page*). Spawning behaviour of sockeye salmon observed in a large aquarium. (E) Male and female spawning. Both are crouching inside the nest with mouths agape and releasing eggs and sperm. (F) Female covering eggs after oviposition while the male stands by. (G) Cross section of a chum salmon redd containing 4 egg pockets, seen through a glass window and a mirror overhead. Water is flowing from left to right. The female is holding over the redd just behind the crest. The size of each window glass is 2.1 × 1.2 m. (H) Both female and male fungus up, loose locomotory control, and die about 9-16 days after spawning and then float downstream.

probe and has lifted herself up. The male drifts back to his original courtship position and may then perform a number of cross-overs. The close temporal association of probing and quivering suggests that probing serves as a signal to the male of the increasing readiness of the female to spawn (Tautz and Groot 1975).

### *Oviposition and Covering of the Eggs*

The behaviour that culminates in oviposition often starts in the same way as a probe-quiver sequence, but instead of probing the female will go into a "crouch" with mouth agape and the male will assume a similar position next to her (Figure 18, Phase 4; Figure 19E) (Tautz and Groot 1975). At this point the gametes are released with both partners vibrating their caudal peduncles and anal and caudal fins. Often, the female will adopt the crouching posture several times in succession before she finally releases eggs. Each time the female crouches the male follows her in a similar posture. However, the male will only release sperm when the female extrudes the eggs.

Within seconds after egg deposition the female moves slightly upstream, turns sideways and gently flexes her tail upwards from the gravel once or twice (Figure 18, Phase 5; Figure 19F). The first few "covering digs" normally do not move gravel but tend to drive the eggs down and into the gravel interstices. Subsequent digs become more vigorous and are repeated almost as rapidly as the female can return to the nest (Tautz and Groot 1975). Occasionally females attack or chase their sexual partner after spawning, especially when he blocks or interferes with the nest-closing process.

Nest closing activities of the female generally last from 15 to 30 min after which time the nest depression is filled. The behaviour then slowly changes into normal digging (Figure 18, Phase 2 and 3), which results in the preparation of the second nest in front of the first one. Most salmonid females dig several nests in succession before they are spawned out.

In Arctic charr the spawning act occurs the same way as described above, but then both fish start to swim rapidly forward and upward while continuing the release of sexual products (Fabricius and Gustafson 1954). The charrs in general appear to perform spawning acts in groups of up to five acts. After a group of spawning acts the female performs slow, rather snake-like undulating body movements while gliding over the bottom of the nest pit and sweeps the gravel with her caudal, anal, and pelvic fins. She stays in an upright position but with her head and tail slightly tilted down as in searching behaviour. These undulating movements can last for up to 56 min before actual cover-digging starts, which covers the nest with gravel (Fabricius and Gustafson 1954; Leggett 1980; Martin and Olver 1980). Such cover-digging has not been observed in lake charr, which only perform the undulating movements (Martin and Olver 1980).

After the spawning act most salmonid males move on, either up- or downstream, in search of females in the process of nest construction. In sockeye and pink salmon, males try to move on after spawning. However, the dense concentrations in which these two species spawn make it often impossible for the males to go anywhere without being attacked from all sides. Therefore, they quickly return to the original female.

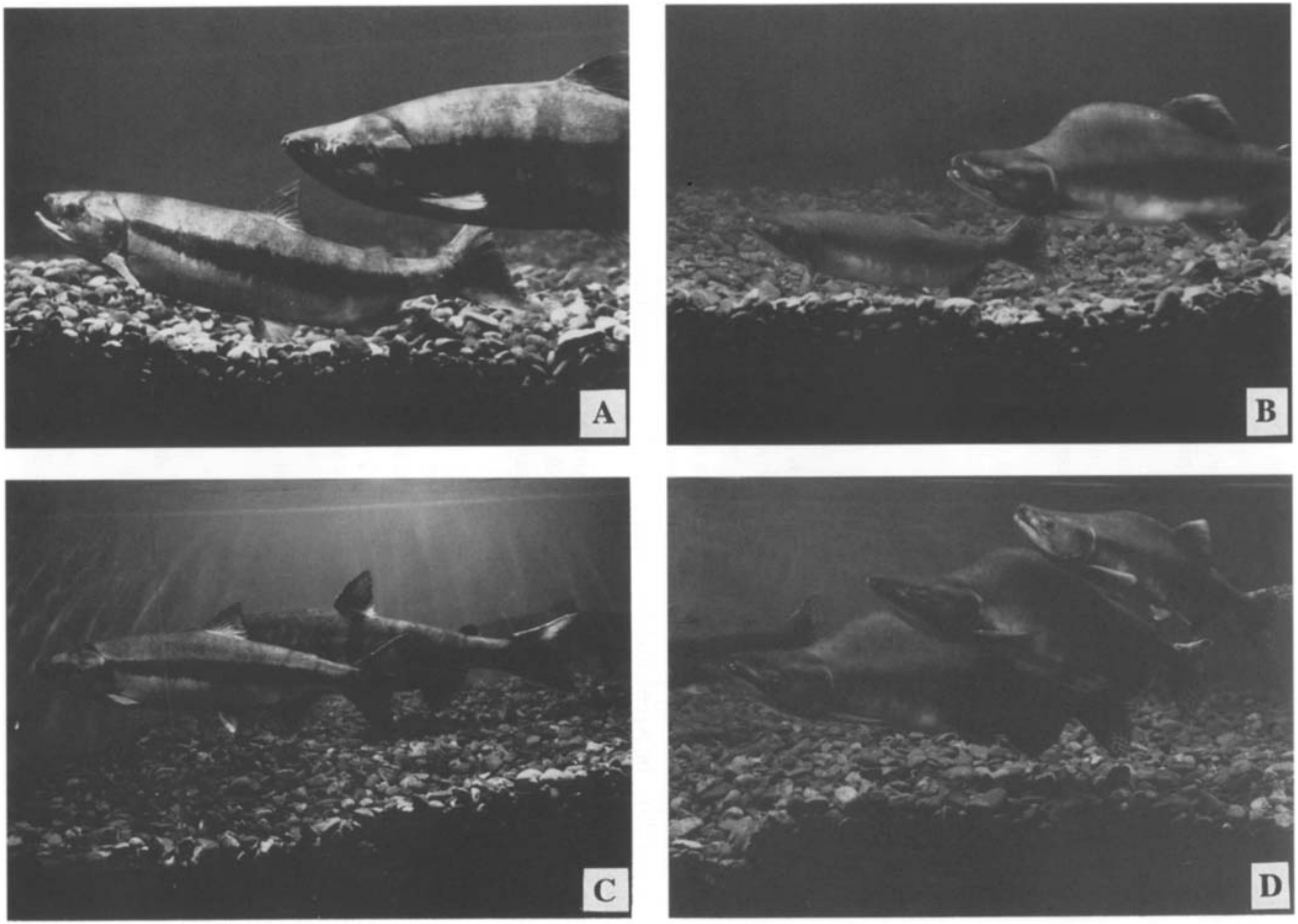
Chum, pink, coho and chinook salmon, rainbow and cutthroat trout, and Atlantic salmon spawn at any time during the day and night (Jones 1959; Tautz and Groot 1975; C. Groot, unpubl. data). Activity is more associated with time to next spawning than with the diurnal cycle (Tautz and Groot 1975). Arctic, brook and Dolly Varden charr spawn primarily during daylight hours (Fabricius 1953; Leggett 1980; Power 1980), whereas sockeye salmon and lake charr mainly spawn after dark (Martin and Olver 1980; C. Groot, unpubl. data).

### *Colour Patterns*

During the spawning season the body colours change. Scales in Pacific salmon are absorbed around the time of freshwater entry and skin pigmentation in all species increases dramatically, with red, yellow, green, purple and black hues as the most prominent shades. In Pacific salmon, when males and females are in full spawning dress, the underparts become darker than the upperparts. This emphasizes roundness (Figures 19 and 20). In Arctic and lake charr, roundness is emphasized in a different way. In these species, the head, back and sides become darker or lighter during the spawning period. When the male begins to court a female, he develops a dark-coloured longitudinal stripe on the side and the back becomes so light that in some specimens it looks almost white. Such colour changes occur only occasionally in females (Fabricius and Gustafson 1954).

The biological significance of the colour changes, in combination with the morphological and behavioural changes, is to advertise sexual readiness and dominance in the males and to render display activities more pronounced. The colours may also aid in species and sex recognition and facilitate social interactions between male and female. The following examples are from Pacific salmon, especially chum and pink salmon, to illustrate some of the principal characteristics of colour patterns in social behaviour. In many cases these apply to other salmonids as well.

Dominance rank in male chum and pink salmon is expressed in several conspicuous ways. Besides changing body shape and behaviour, males develop a vertical stripe or a horizontal bar colour pattern on their lateral sides, or a combination of both (Figure 20A, 20B, 20C and 20D). In chum salmon, the stripe colour pattern consists of black and red-purple streaks that run perpendicular to the length of the fish against a mottled green-yellow background (Figure 20A). In pink salmon the dark green-brown


A
B
C
D

colour above the lateral line continues into a blotchy purplish pattern below the lateral line (Figure 16B and 16D).

The bar pattern consists of a single black band that runs the length of the fish, which is sometimes bordered by a fine white band on top and bottom that enhances it (Figure 20A, 20B, 20C, and 20D). The bellies of male chum salmon are generally black when they have a stripe pattern and white when they have a horizontal bar along the side of the body (Figure 20A and 20C).

The patterns can change rather quickly (within 10 to 15 s) from one to the other and indicate social status (Schroder 1981; C. Groot, unpubl. data). The stripe pattern denotes dominance and is usually very pronounced in big aggressive bucks, whereas the bar pattern occurs in smaller, subordinate males (Figure 20C and 20D). The bar pattern of the latter males resembles that of the spawning female (Figure 20A and 20B) and can be considered a form of "female mimicry" that tends to reduce attack by dominant males. Small subordinate males also resemble females in that they do not possess strongly hooked beaks or large teeth and have a smaller hump on their back (Figure 20C and 20D) (Schroder 1981; C. Groot, unpubl. data).

Dark lines running perpendicular to the long axis of an object tend to magnify its width, while those running parallel to the long axis make it appear longer and reduce its bulk. The stripe pattern on dominant males enhances the apparent width or size of a male and the bar on territorial females and subordinate males may disguise their size and thus be less threatening to conspecifics. By avoiding being attacked by other males, "female mimics" are able to position themselves next to females during spawning and thus fertilize eggs by "sneaking". In general, females tend to delay nest construction when being courted by socially subdominant and relatively small males and will often chase such males away (Schroder 1981; C. Groot, unpubl. data).

Pacific salmon females have the same colour pattern differences as males but less distinct. In the early stages of freshwater residence they exhibit the stripe pattern but

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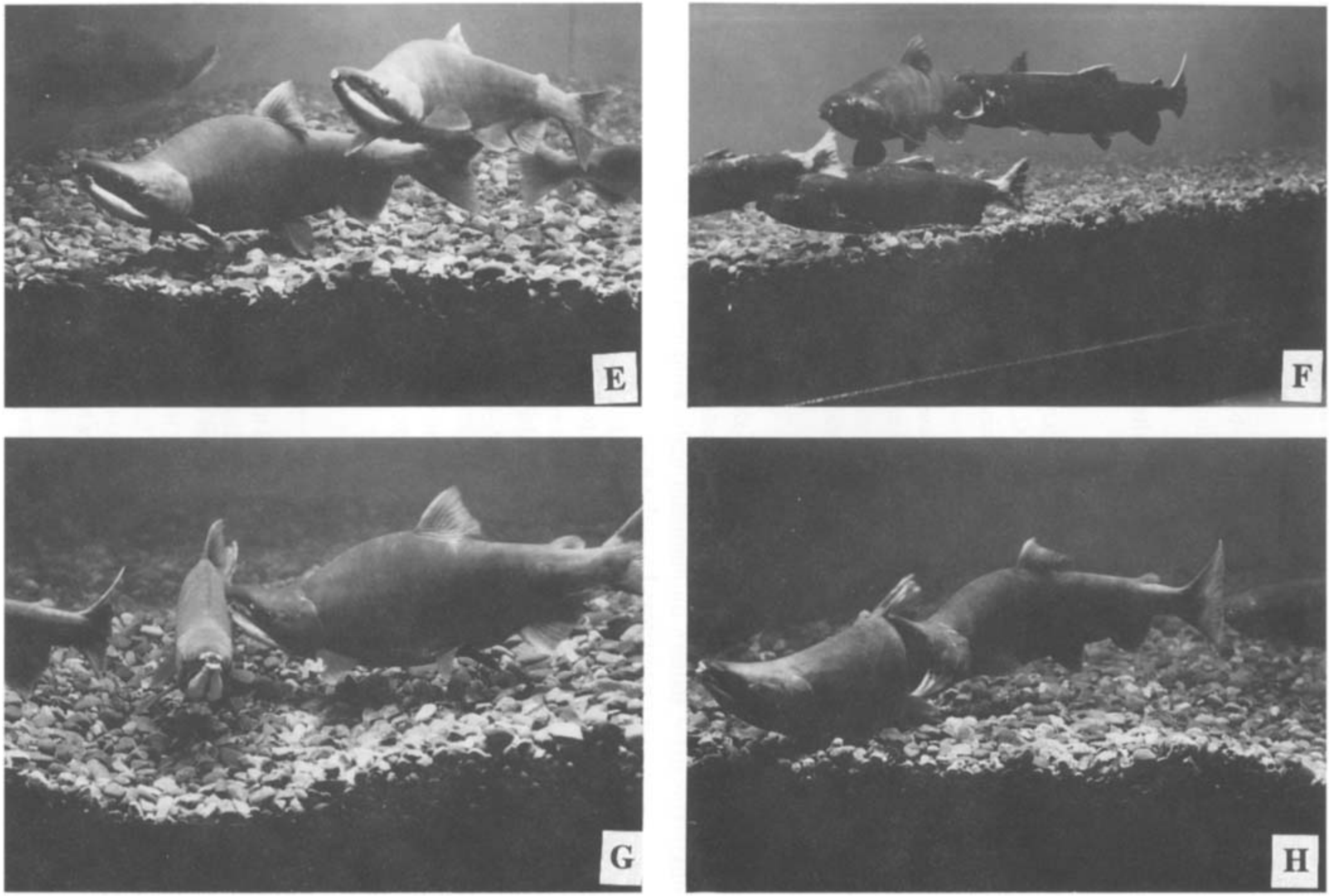
FIGURE 20 (*opposite page*). (A) Female and male chum salmon during spawning displays. The female is probing the nest and has a distinct horizontal stripe, whereas her partner shows the vertical bar pattern of a dominant male. (B) Same as A for pink salmon. The male in this case is a subdominant fish as indicated by the obvious stripe. (C) Lateral threat display between two chum males. The one in front shows an obvious black stripe and light belly and was the subordinate among the six males in the aquarium. (D) Three pink salmon males jockeying for position while attending a female constructing a nest. In the male hierarchy, the centre male is the dominant alpha male (no stripe). The male near the female is a subdominant (vague stripe) and the small male closest to the glass is the subordinate (distinct stripe).


E
F
G
H

once construction of the first nest begins, they always develop a dark lateral bar, which they retain until death (Figure 20A and 20B) (Schroder 1981; C. Groot unpubl. data).

### *Agonistic Behaviour*

Agonistic interactions among courting pairs are mostly directed towards members of the same sex. The most intense aggressive encounters take place when a male intrudes on a spawning pair (Figure 20E). Male agonistic behaviour includes overt attacks, bites, grabbing, shaking and threats. It is used to defend nest territories or to chase sexual rivals away from potential mates. If intruders stand their ground then both males will perform "lateral displays" by swimming parallel to each other, with dorsal fins fully raised (Figure 20E). The inside of the mouth in chum, coho, chinook, masu, and most salmonids are brightly patterned with black and white on upper- and lower jaws, tongue, and lips (Shaw 1994). These males will threaten with their mouths slightly opened (Figure 20F). In sockeye and pink salmon, the display patterns are on the underside of the lower jaw and males from these species threaten with their jaws closed and with the snout turned up to display the lower jaw patterns (Figure 20D, 20E and 20G).

While swimming side by side in lateral display, one of the males usually moves forward and then turns perpendicular to the current (Figure 20G). During this "T-display" the current pushes the broadside of the square standing male towards the nose of the downstream rival, who may then attack the side (Figure 20H) or move sideways over the tail of the threatening fish. The latter will then quickly turn 180° to display the other side of the body. These T-display activities may be repeated a number of times before the two fish continue their parallel swimming again.

During an attack a male charges towards his rival with jaws opened widely and tries to grasp a fin or portion of the opponent's body, often the caudal peduncle (Figure 20H). Rapid back and forth head shaking occurs if he gets hold of the opponent. After an attack both males return to the nest site of the contested female and generally resume

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FIGURE 20 cont'd. (*opposite page*). (E) A spawning pair of sockeye salmon being disturbed by intruding male. Males are showing lateral threat display behaviour with closed mouths and head slightly turned up, while parallel swimming. The female is leaving the nest site. (F) Coho salmon males in threat display. The smaller male has opened his jaw, showing the black and white pattern inside his mouth. (G) Sockeye salmon males in threat display. Following parallel swimming the male facing the glass window (male a) has moved squarely in front of an opponent (male b) and is pushing him to the right with the current. Following, male (b) then flips over the tail of male (a), who then quickly will turn 180° clockwise to put himself again squarely in front of male (b). (H) Resident male attacking an intruder with full force. The female is waiting in background until the fight is settled.

lateral display (Figure 20E). A sequence of displays and attacks may last for several hours when the males are of equal strength and size. Generally, however, confrontations among combatting males are settled rather quickly with the resident male driving the intruder away.

The outcome of aggressive contests can be predicted by changes in the colour patterns of the males. At the onset of such battles both males usually possess the vertical stripe pattern. However, during the later stages of a confrontation the weaker of the two males develops the beginning of a horizontal bar. At the end of a confrontation the subdominant male may position himself behind the alpha male and become a satellite to the courting pair (Figure 20H). The colour pattern of satellite males is often a mixture of a bar and stripes. Several satellite males may attend a courting pair at high spawner densities, and the combinations of bar and stripe patterns reveal their status on the dominance hierarchy scale (Figure 20F).

Aggression within pairs is rare (Schroder 1973). However, females attempt to drive subordinate males away by nipping, chasing, or biting. Also, females attack other females when establishing their nest territories and later when they are covering and protecting the redds after all eggs have been buried.

### *Satellite Males*

During the spawning act the female and alpha male lie tightly together with mouths widely agape. If satellite males are present, the most dominant one will position himself in a similar manner on the opposite side of the female. Additional satellites either lie adjacent to these males, or more commonly, attempt to push themselves underneath the female. In these attempts they generally dislodge the female from her nest before egg deposition occurs. The female will then turn around and possibly dig and probe a few times. The males return to their respective positions beside and behind the female, waiting for her to crouch again. Satellite males can fertilize up to 25% of the eggs. The closer a male is to the female the more eggs he will fertilize. In general "large" males have a reproductive advantage over relatively smaller rivals (Schroder 1981).

### *Redd Defence by the Female*

Once a female has completed all of her nests, she covers them under a single mound of gravel, called a redd (Figure 18, Phase 6; Figure 19G). To accomplish this, she performs prolonged and curving diggings of up to 10 or more body flexures. After each digging bout the female returns to a position just behind the highest point of the mound and holds (Figure 19G). She will actively defend the redd area against other females and especially attacks females that are digging nearby. As females of Pacific salmon become weaker during the following days, their skin gets covered with large white (fungus) patches. Eventually such fish are unable to hold position against the current and float downstream to die (Figure 18, Phase 7; Figure 19H). The average time between the start of preparation of the first nest and dying of Pacific salmon for both females and males is about 9 d (range 6-12 d) (Groot and Margolis 1991). In salmonids other than Pacific salmon, part of the spawning population may survive spawning to return during subsequent years.

## THE PACIFIC SALMON

There are seven species of Pacific salmon. Sockeye, pink, chum, chinook and coho salmon reproduce on both the North American and Asian continents, whereas masu and amago salmon occur only in Asia (Groot and Margolis 1991). Pacific salmon are primarily anadromous or have anadromous ecotypes. There are three systems in use to designate the age of Pacific salmon and the time spent in fresh and salt water; the European, the Gilbert and Rich, and the Russian systems (Groot and Margolis 1991). The European system will be used in this chapter; it uses two numbers separated by a period to designate age. The first number identifies how many winters were spent in fresh water by the juvenile after emergence (0., 1., 2., 3.) and the second number indicates the winters spend in salt water by the immature before returning to spawn (.0, .1, .2, .3). Thus, an age 1.2 fish is 4-yr old, counting the time from egg to adult, and has spent one winter in fresh water before migrating to salt water, and returned to spawn after two winters in the ocean. An age 0.3 fish is also 4-yr old but migrated to sea soon after emergence and has spent three winters in the ocean (*see* Table 2).

To save space the descriptions of colour have been omitted in the following species descriptions. Salmonid are very often brightly coloured in striking patterns, especially during breeding seasons. More often they show camouflage markings and colour. Full descriptions of salmonid appearance can be found in the references given in the following sections and in: McPhail and Lindsey (1970); Scott and Crossman (1973); Groot and Margolis (1991); Behnke (1992).

The food of salmonids as noted above is varied, but shows much similarity when species are compared. Salmonids are opportunistic predators and their diet reflects the abundance of prey organisms in their particular habitat (e.g., insects and insect larvae in streams and lake shores; lake zooplankton and fish in the lake pelagic zone; zooplankton, macro-zooplankton and fish or squid in the oceanic pelagic zone). A recent review by Higgs et al. (1995) thoroughly explores this subject.

### *Sockeye Salmon*

Sockeye salmon are economically the most important of the Pacific salmon and, in numbers, are third in abundance behind pink and chum salmon (Figure 21) (Burgner 1991). They are more numerous in the eastern than in the western Pacific, especially in western and central Alaska (Figure 22). Sockeye salmon typically spawn in tributaries and outlet streams adjacent to lakes and the young remain in nursery lakes for one or more years before migrating to sea as smolts. In the ocean they perform extensive migrations and after several years in salt water, return to their lake and stream of origin to spawn.

Sockeye salmon have the most complicated migratory patterns among Pacific salmon. This results from different combinations of freshwater (ranging from none to five winters) and saltwater (ranging from one to six winters) residence times (Table 3). Although primarily an anadromous species, sockeye also occur as a landlocked form, the kokanee, which is a much sought after sport fish. In total, 4-year-old sockeye salmon perform about 12 habitat changes during their life cycle (Figure 11). Sockeye salmon of older age make additional habitat changes as they migrate several more times between overwintering grounds and summer feeding grounds.

*Ecotypes*. The homing ability of sockeye salmon is well documented and straying is generally 2% or less (Quinn 1984, 1985). This high fidelity to the home breeding stream causes many of the stocks to be reproductively isolated from each other. Over time, this has led to the development of many different sub-populations or stocks with genetically distinct characteristics, which are closely adapted to the ecological conditions and constraints of the habitats they live in. The more important variations in the life cycle of the sockeye salmon are: Fry may move directly to salt water after emerging from the gravel; juveniles can spend 2 to 3 yr in lake nursery areas before migrating to sea as smolts; maturing adults may return to their home river after 1-3 or more years in the ocean; spawning can occur in inlet or outlet streams, or along lake shores, which results in different migratory patterns of the resulting fry; ocean fish can return after one winter in salt water as jacks; and the whole life cycle can be completed in fresh water as in the case of kokanee.

In the Fraser River drainage system of British Columbia, the majority of sockeye salmon mature in their fourth year. Thus, the main stream of reproduction flows from a given year to the fourth year following, so that many nursery lake systems have four ecotypes or reproductive lines. One of the lines often considerably exceeds the other three lines in abundance and is called the "dominant line". This phenomenon results in an imbalance in population size, producing a 4-yr cycle of abundance and catch.

*Geographical Distribution*. Sockeye salmon on the North American continent range from the Columbia River northward to Kotzebue Sound in Alaska. On the Asian side of the Pacific Ocean, sockeye salmon populations are found from Cape Chaplina,

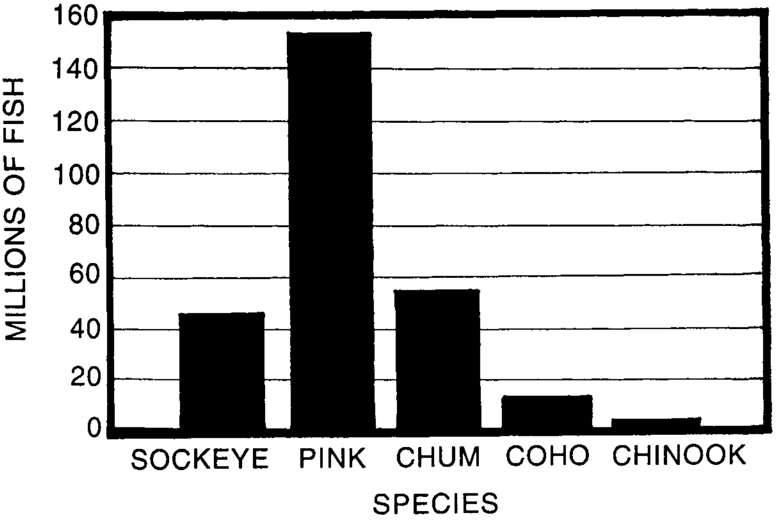

FIGURE 21. The average yearly Pacific salmon catch of Pacific Rim countries from 1977-1986 (INPFC 1979-1988).

southward along eastern Kamchatka, the Kuril Islands, the north coast of Hokkaido, and along west Kamchatka to the north coast of the Sea of Okhotsk (Figure 24A). Some streams on the Komandorskiy Islands have small populations of sockeye salmon (Burgner 1991).

The important production areas for sockeye salmon in North America and Asia are shown in Figure 22. The Bristol Bay area is the most significant sockeye salmon production area in Alaska and includes the extensive lake rearing areas that are part of the Ugashik, Egegik, Naknek, Alagnak, Kvichak, Wood, Nuyakuk, Igushik, Snake, and Togiak river systems (Burgner 1991). Up to 60 million sockeye salmon have returned to this system yearly in the late 1980s and early 1990s.

In southern and northern British Columbia, the Fraser, Skeena, and Nass rivers are the most significant watersheds for sockeye, with spawning populations distributed over a great variety of nursery lakes (Figure 22). Important sockeye salmon runs also occur in Rivers and Smith inlets, where adults spawn in tributaries of Owikeno and Long Lake, respectively. Despite their small size, these two lakes are the most productive in the world in production per unit area (Aro and Shepard 1967). On the Russian side most sockeye salmon are produced in rivers of east and west Kamchatka, especially the Ozernaya River in southwestern Kamchatka and the Kamchatka River along the east coast of the peninsula (Figure 22) (Burgner 1991).

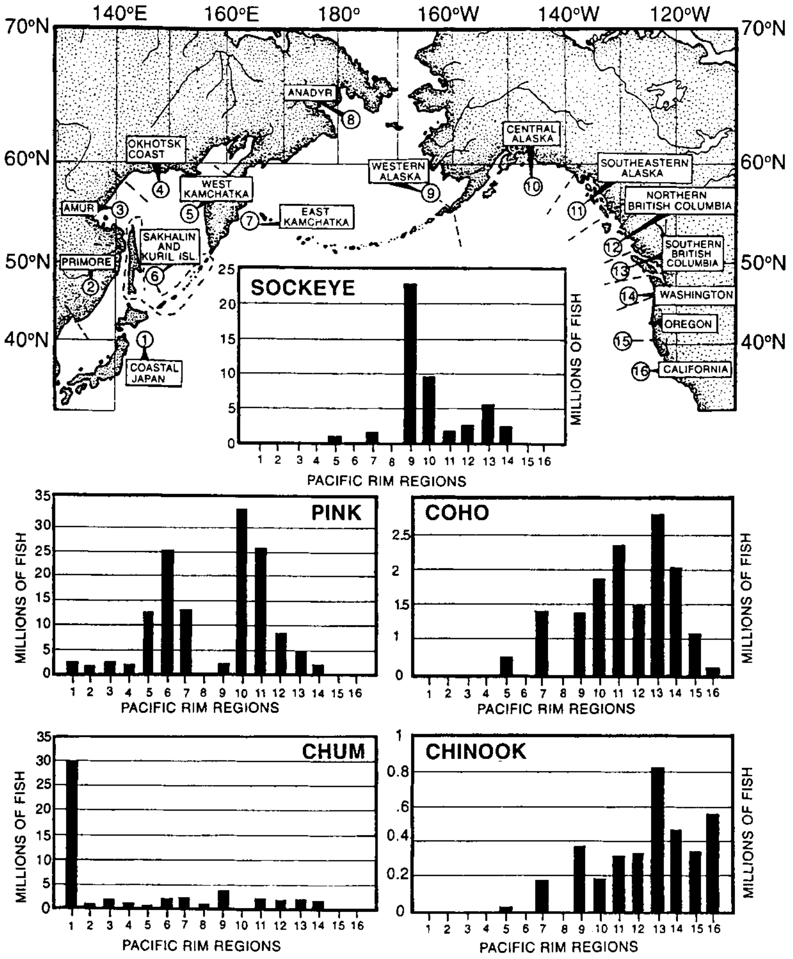

FIGURE 22. The average yearly Pacific salmon catch for 16 Pacific Rim areas from 1977-1986 (INPFC 1979-1988).

*Age and Size.* Sockeye salmon maximally become 7 yr old from egg to spawning adult, with 4, 5, and 6 yr as the most common ages. Within this time span differences occur in time spent in fresh and salt water resulting in a total of 18 age classes (Table 3).

Sockeye salmon returning to spawn from the ocean feeding grounds weigh on the average between 2.5 and 3 kg in the northern areas of their distribution (Figure 23). In more southern areas they weigh less, just over 2 kg in Washington state and slightly over 1.5 kg in Oregon (Figure 23). Substantial variations also exist in mean length (mid-eye

**TABLE 3**

Ranges of ages at maturity for 7 species of Pacific salmon; most commonly occurring ages for each species are encircled.

| Species | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 1.0 | 1.1 | 1.2 | 1.3 | 1.4 | 1.5 | 1.6 | 2.0 | 2.1 | 2.2 | 2.3 | 2.4 | 2.5 | 3.0 | 3.1 | 3.2 | 3.3 | 3.4 | 4.0 | 4.1 | 4.2 | 4.3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amago | | | | | | ⓧ | x | | | | | | ⓧ | | | | | | x | | | | | x | | | |
| Masu | | | | | | x | ⓧ | ⓧ | x | | | | x | ⓧ | ⓧ | x | | | x | x | x | x | | | x | | |
| Coho | x | x | x | | | x | ⓧ | x | | | | | x | ⓧ | | | | | | x | | | | | x | | |
| Chinook | x | ⓧ | ⓧ | ⓧ | ⓧ | | x | x | x | x | x | x | | x | x | x | x | x | | | | | | | | | |
| Sockeye | | x | x | x | | | ⓧ | ⓧ | ⓧ | x | | | x | x | ⓧ | ⓧ | x | | x | x | x | x | x | x | x | x | x |
| Chum | x | ⓧ | ⓧ | ⓧ | x | | | | | | | | | | | | | | | | | | | | | | |
| Pink | ⓧ | | | | | x* | | | | | | | x* | | | | | | | | | | | | | | |

* Great Lakes

to fork of tail) among sockeye salmon populations. In Alaska the mean length for 1.2 females ranges from 45 to 54 cm, and of age 1.3 females from 51 to 59 cm (Burgner 1991). In British Columbia sizes of female sockeye salmon range from 52-60 cm in 1.2 and 1.3 age classes and in males, from 38-66 cm in 1.1 and 1.3 age classes (I.V. Williams, Pacific Biological Station, Nanaimo, B.C., Canada, pers. comm.).

*Upstream Migration.* The timing of return to the home river is specific for different stocks and generally varies very little between years. It is related to river conditions, water flow, temperature at sea and of the river upon entry, distance to the spawning grounds, and temperature in winter during incubation (Burgner 1991). The latter condition results in a general trend of earlier upstream migration in the season from south to north over the distribution range.

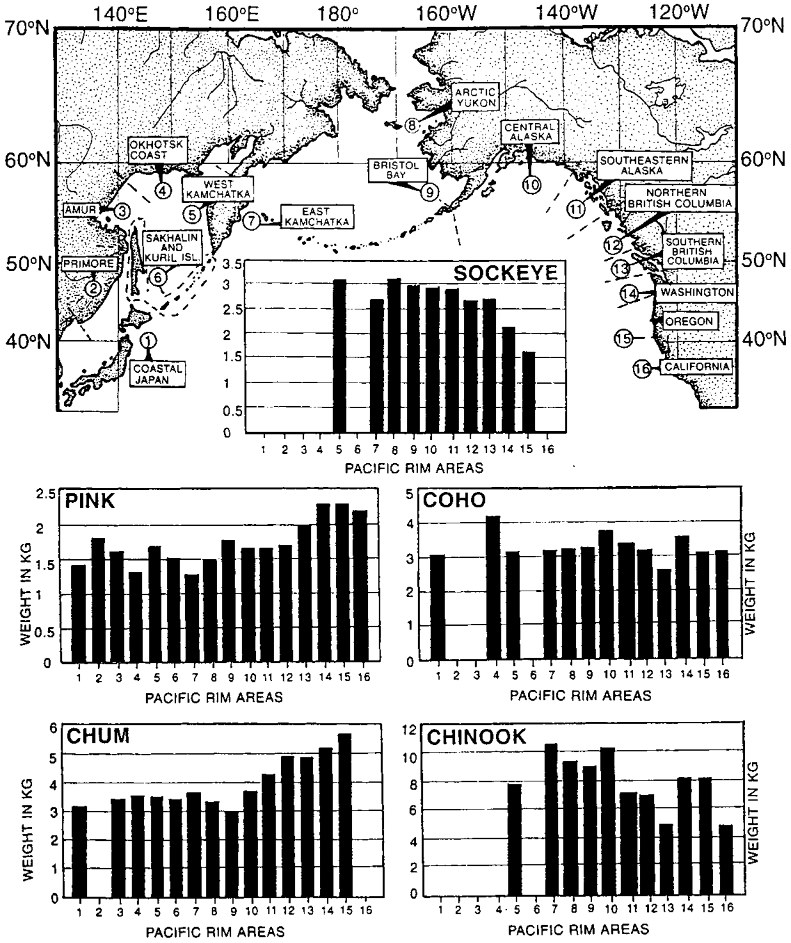

FIGURE 23. The average annual weights of Pacific salmon for 16 Pacific Rim areas from 1977-1986 (INPFC 1979-1988).

Within a river system, stocks that have to migrate farthest and that spawn in coldest water conditions return to the home stream earliest in the season. Stocks that have to migrate shorter distances and spawn in generally warmer streams approach coastal areas later in the season to commence their upstream migration. Some coastal stocks (e.g., from Great Central and Sproat lakes on Vancouver Island) spawn short distances from the coast but migrate upstream early in June and July, and then stay for several months in the lake before spawning in late autumn. In these cases the river often reaches temperatures of 20°C or higher during the summer months.

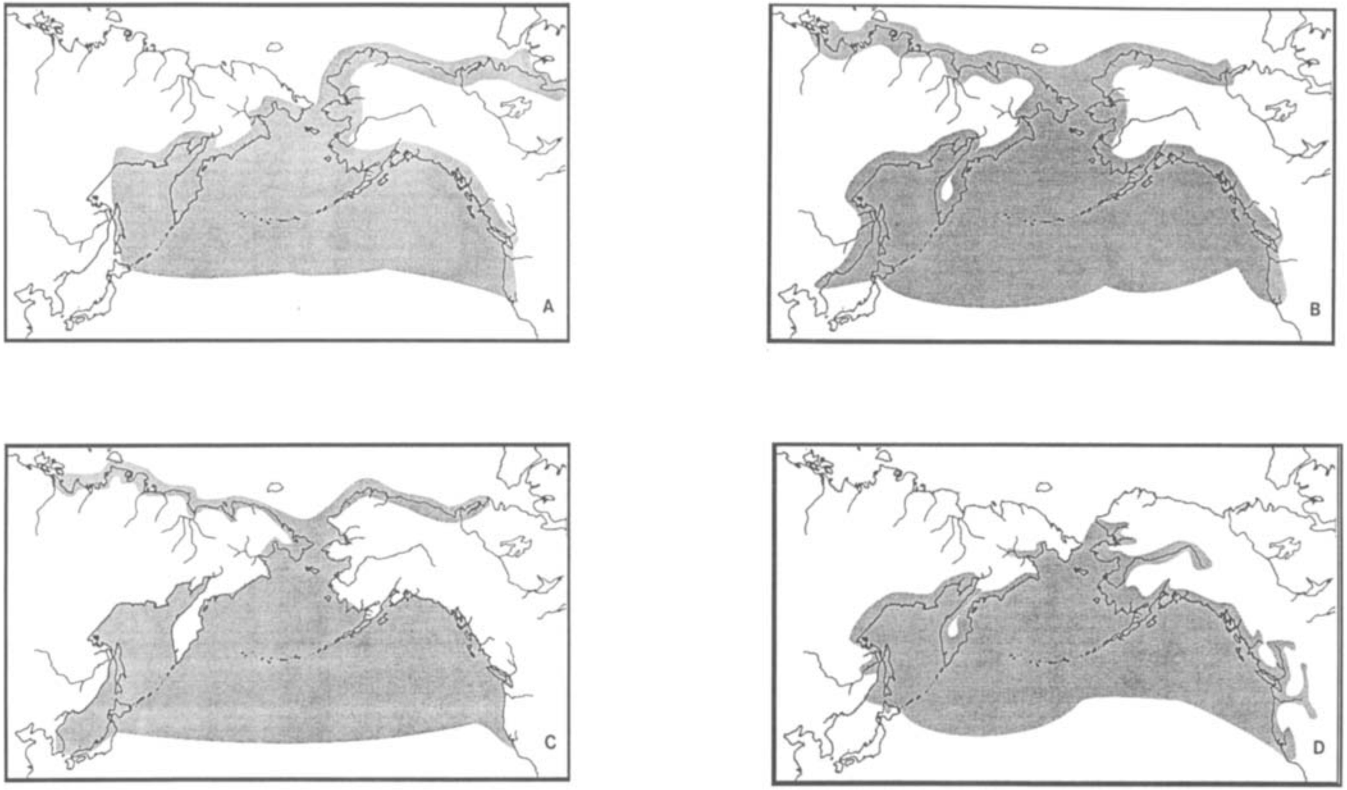

FIGURE 24. Geographic distribution of (A) sockeye salmon, (B) pink salmon, (C) chum salmon, and (D) chinook salmon.

Migration upstream in large rivers, such as the Fraser River, generally occurs along the banks of the river and close to the bottom (T. Mulligan, Pacific Biological Station, Nanaimo, B.C., Canada, pers. comm.). Movements can be rapid, averaging more than 30 km/d (Burgner 1991). Upon reaching the nursery lakes sockeye salmon may hold in large groups off the outlet of the spawning tributary or in pools in the river itself to complete the final stages of maturation.

*Reproduction.* After sockeye salmon have reached the breeding grounds they tend to hold in aggregations for some time, quietly maintaining position in the deeper parts of the river. First, the males start to become aggressive. They perform lateral threat displays, primarily at the edge of the aggregations, and as a result of the agonistic activities move out of the holding groups. Some appear to occupy temporary territories around the groups and defend these areas against others for a few days. Others leave the groups and roam over the spawning grounds. Soon the females also become restless and start to react aggressively towards each other. This results in a spacing out of the females and eventually many leave the group and move to the spawning grounds where they patrol the gravel beds in search of a place to spawn. The subsequent nest digging behaviour and courtship displays follow the pattern described above in the section on Spawning Behaviour.

In British Columbia, spawning takes place in late summer in northern and central areas, such as the Nass and Skeena rivers and Rivers and Smith inlets, and in autumn in the more southern Fraser River and Barkley Sound river systems. The timing of spawning is related to the temperature regime of the stream, which in turn determines hatching and emergence time. There is a critical upper limit of 20°C, above which spawning will not occur (I. V. Williams and K. Hyatt, Pacific Biological Station, Nanaimo, B.C., Canada, pers. comm.).

Sockeye salmon show a diel rhythm in their spawning activities. During the morning male and female fish primarily defend the nesting site. This involves intensive agonistic and overt aggressive activities, because spawning occurs in dense concentrations, which locks the fish into a tight mosaic pattern of nest territories. In addition, unattached males roam around in search of mates and females cruise the spawning grounds searching for a nest site. In the afternoon the female prepares the nest, while the male continues to fend off intruders and attack and chase trespassers. Actual spawning occurs primarily after darkness has set in until about midnight. It appears that only one spawning per night occurs. During the following days the pattern of defending a territory in the morning, preparing the nest in the afternoon, and spawning at night is repeated three to four times until all the eggs have been shed.

The male attempts to leave the nesting area after spawning when the female is filling in the nest pocket. But as soon as he ventures outside the nest site, he is challenged by neighbouring territory holders and quickly returns to the original female. Sockeye salmon pairs, therefore, tend to stay together for the total spawning period of about 7-9 d until the female has laid all the eggs (C. Groot, unpubl. data). When the female is spent, she continues to finish the redd and defends the area against females searching for nest sites and males that are passing by. She also attacks neighbouring females, especially when they are performing a digging bout. Nine to ten days after starting to spawn, male and female die. If agonistic activities on the spawning grounds have been intense in situations of high spawning densities, females show varied levels of egg retention when they die (C. Groot, unpubl. data).

The fecundity of sockeye salmon females averages about 2,000-2,400 eggs. In some areas of Kamchatka fecundity can be as high as 5,000 eggs per female (Burgner 1991). The smaller kokanee females have from 300 to 2,000 eggs. Sockeye salmon eggs are dark orange-red in colour and relatively small in size compared with those of other Pacific salmon. Egg diameter ranges about 5.3-6.6 mm.

*Incubation and Emergence.* The average degree-days from fertilization to emergence for sockeye salmon ranges from 851 at 4°C to 1,090 at 10°C (*see* Chapter 5). The alevins stay in the gravel for varying amounts of time after hatching and then emerge as fry from the gravel at night. Fry migration generally peaks before midnight with sometimes a small peak before dawn. The first activity of the fry after emergence is to fill the swim bladder by gulping small bubbles of air. This allows them to maintain position anywhere in the water column by adjusting the gas content in the swim bladder.

*Juveniles.* The movements of sockeye salmon fry originating from populations spawning in tributaries and outlets differ. In both cases the fry initially operate as individuals after emergence. Tributary fry move actively downstream when leaving the spawning gravel, whereas outlet fry attempt to reach the river banks as soon as possible and then swim upstream to the lake (McCart 1967; Brannon 1972).

In most instances the journey downstream from the tributary spawning grounds to the lake is completed in one night. If the lake is not reached during the first night, the fry hide in the gravel during daylight hours and rise at night again to continue their downstream migration (McDonald 1960). In the lake the behaviour of the fry changes. The fish abandon their solitary habits, become positively rheotactic, form schools, and swim and feed in brightly lit areas. They remain along the shores of the lake for a short period, probably a few days, and then move offshore and descend to deeper water levels to take up a pelagic life (McDonald 1969). In some nursery lakes, fry may move appreciable distances during the summer and autumn (McDonald 1969). Sockeye salmon fry originating from outlet spawning grounds tend to swim across the current to the river bank. Along the shore the fry collect in large groups and rapidly change their behaviour. They become day active, form schools, feed in the open, and actively swim upstream along the river banks towards the lake.

In the open waters of the lake, sockeye salmon juveniles perform diel vertical movements, rising up into the surface layer at dusk, remaining there throughout the night, and descending at dawn to a daytime level of 16 m or more (McDonald 1969; Levy 1987). The extent of vertical migration varies among lakes depending upon lake conditions. In lakes with much glacial silt, the diel movements are much less pronounced and the fry tend to stay closer to the surface (Levy 1987). The pelagic existence of the juveniles persists until the following spring when they transform into smolts and migrate out of the lake downriver to the estuary.

*Smolts*. Downstream migration of sockeye salmon smolts commences in spring when surface water temperatures reach 4-4.5°C (Foerster 1968). In northern lakes, such as Babine Lake in British Columbia, this temperature range coincides with ice break-up. In some years, however, ice breaks up later than usual, in which case the smolts commence their migration under the ice (Groot 1965). It seems that smolts will wait for the preferred environmental conditions, but if these conditions do not occur within a limited time period, migration will proceed (Foerster 1968).

Smolt migration in many nursery lakes ceases when surface water temperatures reach 10°C (Foerster 1968). Warm surface waters appear to create a temperature blanket through which the smolts will not pass. Ward (1932) proposed that this is the origin of residual salmon. Foerster (1968) hypothesized that it may explain the occurrence of 2-year-old smolts because small sockeye salmon, which have a tendency to migrate later in the season, may be blocked from moving out of the lake by a temperature blanket during the end of their first year.

*Ocean Phase*. Upon reaching salt water in May and June, sockeye salmon smolts from Pacific Northwest areas first migrate northward along the coast lines of British Columbia and southeastern and central Alaska in a narrow band (Hartt and Dell 1986; Groot and Cooke 1987). According to Welch et al. (1995) the juveniles then overwinter in waters below 7°C in the North Pacific Ocean. With the coming of spring the sockeye move south and are found up to the 15°C isotherm by summer (Margolis et al. 1966; French et al. 1976; Welch et al. 1995).

Sockeye salmon smolts from the Bristol Bay area move west to southwest upon leaving fresh water and then move through the passes between the Aleutian Islands into the Gulf of Alaska. On the Asian side, sockeye salmon smolts migrate southward along the shores of Kamchatka and then migrate eastward into the North Pacific Ocean to areas south of the Aleutian Islands (French et al. 1976; Burgner 1991). Some sockeye salmon return to spawn after one winter at sea as "jacks", however, most stay two or three winters in the ocean. In the Fraser River the majority of sockeye return as 4-year-olds (age 1.2) after spending two winters in the ocean, whereas in more northern areas returning fish can be a mixture of 4-, 5-, and 6-year-olds.

Sockeye salmon originating from Washington, British Columbia, and southeastern Alaska begin their home migrations by first moving northward from the spring ocean feeding grounds and then turn shorewards towards their natal river estuary (Margolis et al. 1966; French et al. 1976; Groot and Quinn 1987). Upon reaching the river estuary, there can be a short holding period of a few days to several weeks before upstream migration to the parental nursery lake commences. The rate of travel of Bristol Bay or Fraser River sockeye in the ocean during homing, as determined from tag-recovery data, can be between 40 and 60 km/d (French et al. 1976; Hart and Dell 1986; Groot and Quinn 1987). At this rate, the fish must swim day and night for days on end at their optimum sustained swimming speed of about 1 body length/sec (about 2 km/hr) on a very direct course to cover the distance between release and recovery sites (Groot and Quinn 1987). The sockeye salmon that travel shorter distances from their ocean feeding grounds to the home river migrate at slower rates per day (8 to 13 km) (French et al. 1976).

### *Pink Salmon*

Pink salmon are the most numerous of all Pacific salmon in North Pacific Ocean waters (Figure 21). They have historically been more abundant on the Asian side with commercial catches of more than 200 million fish in the 1930s and early 1940s (Heard 1991). More recent harvest rates for Asia and North America are similar with averages between 1977 and 1986 of about 25 million for the Sakhalin and Kuril Islands area, 25 million for Kamchatka, and 26 and 34 million for southeastern and central Alaska, respectively (Figure 22). Pink salmon distinguish themselves from other Pacific salmon by having a fixed two-year life cycle and by migrating to sea soon after emergence from the gravel as fry (Heard 1991). The fixed two-year life cycle results in two separate population lines, i.e., even- and odd-numbered year stocks (Neave 1952, 1966).

*Ecotypes.* The fixed 2-yr life cycle in pink salmon gives rise to two distinct ecotypes, even- and odd-year populations. The Fraser River has predominantly an odd-year cycle of pink salmon, Queen Charlotte Island streams have even-year cycles, and the area between has both cycles. In the latter case two different and unique stocks of pink salmon use the same streams for breeding, each in its own year (Neave 1952, 1966).

*Geographical Distribution.* On the North American continent, pink salmon range from the Sacramento River in central California to the Mackenzie River, which drains into the Arctic Ocean (Figure 24B). Pink salmon have also now become well established in the Great Lakes as a result of an introduction into Lake Superior (Heard 1991). On the Asian side pink salmon are distributed from northern Korea to the Jana and Lena rivers in northern Siberia. In Japan pink salmon spawn in a number of rivers of Hokkaido (Neave et al. 1967; Heard 1991).

During their ocean life phase pink salmon are found throughout the North Pacific Ocean and Bering Sea north of 40°. Main centres of abundance are southeastern Alaska, Kodiak Island, Cook Inlet, and Prince William Sound (Figure 22). In British Columbia, the Fraser and Skeena rivers account for about half of the spawners and an additional 25% is produced in 50 smaller streams. Odd-year pinks are prevalent in the Fraser River system, providing about 70% of the total catch. In even-numbered years, pink salmon are abundant in central (streams near Johnstone Strait) and northern parts of British Columbia and in Alaskan streams. Even-year pinks are also abundant in the Queen Charlotte Islands in a number of medium-sized rivers (Aro and Shepard 1967).

*Age and Size.* Because of their strict 2-yr life cycle, pink salmon generally occur only in one year class (Table 3). In rare occasions, 1-year- and 3-year-old pink salmon are found in natural systems (Heard 1991). The accidental release of pink salmon into the Great Lakes resulted in an unexpected self-sustaining population (Heard 1991), which over the years developed 1- and 3-year-old fish besides the major component of 2-year-olds (Kwain and Lawrie 1981). Pink salmon are one of the fastest growing fish. However, because of their short life cycle, they are the smallest in weight of the Pacific salmon (Figure 23). Between 1977 and 1986 the weight of commercially captured pinks ranged from 1.3 to 2.3 kg. North American pink salmon are in general larger (1.7-2.3 kg) than those of Asian origin (1.3-1.8 kg) (Figure 23).

*Upstream Migration.* River entry of pink salmon occurs mostly during daylight hours between June and September. Spawning grounds can be as far upriver as 700 km, but generally are within 100 km of the coast (Heard 1991). Pink salmon have difficulty jumping falls and in navigating cascades. Upon arrival at the spawning stream pink salmon often remain in bays, estuaries, or other water bodies for up to a month. During this time of final sexual maturation, they swim near the surface and often leap out of the water and turn their bodies sideways before reentering the water (Heard 1991).

*Reproduction.* Mature pink salmon males develop a marked hump and also acquire a large kype. Morphological changes in mature females are only minor but they show the same colour changes as the males. Spawning of pink salmon takes place in autumn from late August to early October. Most pink salmon stocks start defending nesting territories as soon they have moved on to the breeding grounds. Spawning takes place in dense concentrations, primarily in stream areas with riffles. Spawning densities greater than 0.8 females/$m^2$ may affect spawning behaviour (Heard 1991).

Nesting areas border each other in a honeycomb mosaic and vary in size from 1.1 to 2.0 $m^2$. The size of pink nests varies from 60 to 150 cm in width and from 107 to 250 cm in length (Heard 1991). Water levels of pink salmon spawning grounds are from 30 to 100 cm deep with water velocities ranging from 30 to 140 cm/sec (average 60-80 cm/sec). In dry years, spawning can occur in depths of 10-15 cm (Heard 1991). Temperatures during spawning range from 7 to 19°C, with most spawning at 10-12°C (Heard 1991). Male-female interactions during spawning are similar to that described above in the section on Spawning Behaviour.

Nest construction and defence of territories appear to continue day and night, but egg deposition tends to occur mainly at dusk and during darkness (Heard 1991). Many actively courting pairs are attended by a number of satellite males (up to ten), which join the pair in the nest when the eggs are finally shed.

Fecundity of mature pink salmon females ranges from 1,200 to 1,900 eggs, depending on size, region, and year. Specific fecundity for some British Columbia populations increases by 472 eggs per kg in weight (Heard 1991). Pink salmon females deposit an average of two (range, one to four) egg batches per nest, each pocket containing an average of slightly more than 500 eggs. Average depth at which eggs are buried is 20-30 cm (range 15-50 cm). Spawning time of pink salmon females from selection of first nest site to death averages about 10.8 d in southeastern Alaska (range 7-21 days). Deposition of all eggs can occur between 1 and 8 d (Heard 1991).

In areas with heavy runs of pink salmon, females spawn in waves. Up to three waves or more may occur when spawning ground availability is limited and females have to wait until those spawning have finished their redds and died. In such cases total spawning time for a run may last from 1.5 to 2 months (Heard 1991). In some areas pink salmon will spawn in brackish water conditions in river mouths. Intertidal spawning in some short coastal streams can be as high as 74%. In southeastern Alaska intertidal spawning was estimated at about 14% of all spawning (Heard 1991).

*Incubation and Emergence.* Successful incubation is dependent on stream flow, temperature, and oxygen content. Duration from egg fertilization to time of emergence for pink salmon eggs incubated under experimental conditions at 4, 7, and 10°C was 195, 139, and 103 days, respectively (J. Jensen, Pacific Biological Station, Nanaimo, B.C., Canada, pers. comm.; *see* Chapter 5). The average degree-days ranged from 780 to 1,030.

Deformities occur when eggs are incubated at low temperatures (3-4.5°C) during early embryonic development (before gastrulation). Dissolved oxygen levels in the gravel of breeding grounds vary from 5.4 to 10 mg/l. Levels of 3-4 mg/l and lower are lethal or lead to deformities of the embryos. Generally, carbon dioxide ($CO_2$) concentrations range from 8 to 20 mg/l and pH from 5.9 to 6.2 on pink salmon spawning grounds. Freshwater survival from egg to alevin averages 10-20%, but can be as low as 1% (Heard 1991). The fry emerge from the gravel at night, mainly in April and May, and immediately migrate to sea.

*Juveniles.* After emerging, young pink salmon actively swim or passively float downstream (Neave 1955; Hoar 1956). If salt water is not reached during the first night, the fry will hide in the gravel during daylight hours (McDonald 1960). Migration downstream to the estuary is accomplished by a number of nightly "jumps", in which the fry move as individuals. Upon reaching the estuary the behaviour changes. The fry become light-adapted and start to swim around during daylight hours in schools, actively feeding.

Stock-specific variations in the downstream movement patterns of pink salmon fry are evident. In many river systems fry migration is primarily nocturnal as indicated above. However, in some rivers, movement of fry may extend into and throughout daylight hours. The degree of migration during daylight generally increases as the distance that the fry have to travel in the river becomes large and as water turbidity increases (McDonald 1960).

*Ocean Phase.* During the first few months of saltwater residence, pink salmon fry generally form large aggregations in specific places along the shores of inlets and inland seas and move rapidly from one area of aggregation to another (Parker 1965; Healey 1967). Starting in July and continuing into the autumn the aggregates break up, and in North America, juvenile pink salmon start to move northward in a band along the coast of British Columbia and southeastern Alaska (Hartt 1980; Hartt and Dell 1986). A similar phenomenon seems to occur along the coast of Kamchatka but with the juveniles moving southward.

Pink salmon stay in ocean waters colder than 7°C in winter, and then migrate southward in spring before returning to their natal river area to spawn (Welch et al. 1995). Those heading for North American rivers often move north first from the summer feeding grounds and then turn east and southeastward towards the home streams (Neave et al. 1967). On the Asian side the maturing pink salmon, which overwinter near the Aleutian Islands, migrate rapidly and on very direct routes towards the spawning rivers along the west Kamchatka coast (Neave 1964; Takagi et al. 1981). Some pink salmon stocks may stay in near-shore areas during their saltwater residence period, as in Puget Sound, Washington (Takagi et al. 1981).

### *Chum Salmon*

Chum salmon are the second most abundant species of Pacific salmon. Annual commercial catches in coastal areas have ranged from 35 to 65 million fish between 1977 and 1986, and average around 55 million (Figure 21). Chum salmon have the widest distribution of all Pacific salmon and are much more prominent on the Asian than on the North American side of the Pacific Rim. The greatest production occurs in northern Japan (Honshu and Hokkaido), primarily as a result of an extensive and successful hatchery program. The chum salmon's life cycle is relatively simple. The fry generally leave for the sea soon after emergence and the maturing adults return for spawning after two, three or four winters in the ocean. The migrations in the ocean are the most extensive of the Pacific salmon and cover many thousands of kilometres (Neave et al. 1976).

*Ecotypes.* Many regions have chum salmon returning early (summer) or late (autumn) to their home stream, which differ morphologically, physiologically, and behaviourally from each other. Compared with summer chum salmon, autumn stocks have less developed reproductive products at time of river entry, spawn later, are larger in size, and show greater fecundity (Salo 1991). Early run chums generally spawn in the main stems of streams, whereas late spawners occupy smaller streams with springs. The latter provide favourable incubation temperatures during winter (Salo 1991). Although most spawning takes place in rivers, chum salmon also frequently occupy tidal areas and have been observed digging nests in places remote from any surface freshwater streams (Bailey 1964). In this case their life cycle is completed in salt water.

*Geographical Distribution.* Chum salmon are distributed from the San Lorenzo River in Monterey, California, and the Naktong River in Korea in the south, to as far north as tributaries of the Arctic Ocean, such as the Lena River in the west and the Mackenzie River in the east (Figure 24C) (Salo 1991). They are the predominant Pacific salmon species in the far north. Centres of abundance in Asia are northern Japan, the Amur River, the Kamchatka Peninsula, and the Anadyr River (Figure 22). In North America, the main production areas are southeastern Alaska and Prince William Sound, with commercially viable runs returning to Cook Inlet, Kotzebue Sound, and the Yukon River as well as to many streams in British Columbia (Figure 22) (Salo 1991). Occasionally chum salmon have been caught in the Mackenzie River (Dymond 1940).

*Age and Size.* The age of chum salmon can vary from 2 to 6 yr, with ocean periods of 1 to 4 yr before returning to the home river. Most commonly, chum salmon stay in the ocean for 2, 3, or 4 winters (Table 3). In many northern regions 4-yr-olds constitute the predominant group, ranging from 60 to 90% of the population. In more southern regions 3- and 4-year-olds occur in about equal proportions (Salo 1991).

Adult chum salmon can grow as large as 109 cm in length and 21 kg in weight (Salo 1991). In the commercial fishery in coastal areas of Asia weights of chum salmon range from 2.2 to 5.6 kg (mean 3.2-4.4 kg) and in North America from 3.0 to 6.3 kg (mean 3.0-5.6 kg) (Figure 23). The heaviest chum salmon are harvested in British Columbia, Washington, and Oregon waters, averaging 5 kg and larger. Four-year-olds or age-0.3 chum salmon from Asia have an average fork length of 56-78 cm and from North America, of 60-81 cm (Salo 1991).

*Upstream Migration.* When chum salmon arrive in the area of their home stream they often hold offshore in salt water for varying amounts of time where they go through the final stages of maturation and attain full spawning colours. Migration upstream is triggered by an increase in stream runoff early in the season. Later in the season high waters are not as essential (Salo 1991). In Japan, chum salmon enter streams when temperatures drop to 15°C and the majority move upstream at temperatures from 10 to 12°C. Migrations of chum salmon upstream are generally short because the main spawning grounds are mostly in small coastal streams not far from the ocean. Chum salmon do not surmount rapids and waterfalls very well. In larger rivers, spawning is essentially confined to the lower 200 km of the river. However, in some systems, such as the Amur, Yukon, and Mackenzie rivers, chum salmon have been observed spawning as far as 2,000 to 3,000 km from the sea (Dymond 1940; Salo 1991). Spawning generally occurs immediately after arriving at the breeding grounds (Salo 1991).

*Reproduction.* Chum salmon spawn over a long seasonal period. In glacier-fed streams in northern and central coastal regions, mature fish arrive on the spawning grounds as early as July. In southern areas few fish appear before September and in some streams spawning may last well into January. Chum salmon prefer to spawn immediately above turbulent areas, or where there is upwelling. Their spawning behaviour has been described in the section Spawning Behaviour. Schroder (1982) reported that most females (>80%) completed spawning within 30-40 h after starting their first nest in the Big Beef Creek experimental spawning channel. He also found that about 35% of the eggs were deposited in the first nest and that the last few nests contained only one-half to one-quarter of the number of eggs found in the first one. Males remain sexually active for 10-14 d.

The average depth of chum salmon nests ranges from 21.5 to 42.5 cm (Burner 1951). Bruya (1981) found that egg depositions in gravel depths ranging from 20 to 50 cm resulted in high survival rates (mean 84%). Premature emergence of fry occurred in nests less than 20 cm deep and rose to 80% at nest depths of 10 cm. An average of 25% of chum salmon in Hokkaido select gravel for spawning <0.5 cm in diameter, 5% select gravel from 0.6 to 3.0 cm, and 30% select gravel >3.1 cm (Sano 1959). Of chum salmon redds in the tributaries of the Columbia River, 13% consist of gravel larger than 15 cm, 81% have gravel 15 cm or less, and 6% are of silt and sand (Burner 1951). Rukhlov (1969) noted that when the proportion of sand was 22% or greater, the survival of the eggs was <50%.

Chum salmon have large eggs, which can range in diameter from 7.1 to 9.5 mm. Beacham and Murray (1986) reported that egg diameter is correlated with female size and is dependent on spawning time. The fecundity of chum salmon females ranges from 909 to 7,779 eggs in Asia (average means 1,800-4,297) and from 2,018 to 3,977 eggs in North America (annual means 2,017-3,629) (Salo 1991). In Asia, the relative fecundity for northern stocks is higher than for southern stocks. In North America, the relative fecundities of southern stocks are slightly higher than for northern stocks. Salo (1991) assumed that this may be related to decreasing survival rates from south to north in Asia and north to south in North America. As with most Pacific salmons, chum salmon die soon after spawning.

*Incubation and Emergence.* Incubation time and embryo development of chum salmon depend on temperature and vary between summer and autumn stocks. Chum eggs require about 400-600 degree-days to hatch and 700-1,000 degree-days to the yolk absorption stage (Salo 1991). Time of development from egg to emergence for British Columbia chum salmon is 260 d at 4°C, 128 d at 7°C, and 105 d at 10°C (J. Jensen, Pacific Biological Station, Nanaimo, B.C., Canada, pers. comm.). Mortality increases significantly when temperatures are lower than 1.5°C during early development.

Survival rates of chum salmon eggs and alevins decrease rapidly at dissolved oxygen levels less than 2 mg/l. Low oxygen levels during incubation cause reduced larval development rate, growth rate, and egg yolk conversion efficiency, which can result in mortalities, abnormalities, premature hatching, and delayed emergence. Median lethal levels of oxygen rise slowly from fertilization to hatching (Salo 1991).

Chum salmon alevins are photonegative from day 6 to 25 after hatching. This changes rapidly to photopositive behaviour towards the time of emergence. Negative phototaxis during larval development keeps the fish in the gravel and away from predators, whereas positive phototaxis facilitates emergence and swimming to the surface to fill the swim bladder and initiates subsequent schooling (Salo 1991). Emergence from the gravel of chum salmon fry occurs from March to June during the early night time hours. It is earlier in the season in the south and later in the north (Salo 1991).

*Juveniles*. Chum salmon fry generally migrate directly to estuarine waters upon emergence from the gravel. For populations that spawn near coastal areas, the migration to salt water is achieved in one night. Fry from populations that breed far inland, as in some of the large rivers, may spend many days moving downstream, initially in a series of nightly "hops" and then in schools during the day. Migration downstream has been described as a combination of displacement and active swimming, depending on current, temperature, and visual reference points (Hoar 1958). Emerging fry are between 25 and 30 mm long. The first scales appear when the fish are 40 mm and scale development is complete by the time they reach a length of 50 mm (Salo 1991).

*Ocean Phase*. After reaching salt water young chum salmon are generally distributed in schools of varying sizes along shorelines. The juveniles feed actively and grow rapidly. This stage lasts from April to July. By the end of July or early August most of the young leave the water's edge and in North America start to move northward in a band along the coast in the company of pink and sockeye salmon juveniles (Hartt and Dell 1986). In Asia the fry originating in the more northern areas move southward along the east coast of Kamchatka and those migrating out of Japanese streams migrate north and eastward along the coast during late spring and early summer (Sano 1966).

During their first winter at sea chum salmon juveniles occupy the northern part of the North Pacific Ocean in waters colder than 7°C. The following spring and early summer, as ocean waters warm up, the immature fish move southward in the North Pacific Ocean as far as the 15°C isotherm. During autumn and early winter they migrate north again to overwinter in northern waters (Welch et al. 1995).

The maturing chum salmon returning to their home streams along the North American coast move first northward in spring and summer from the ocean feeding grounds and then turn toward the rivers of origin in autumn. Fish that stay in the ocean longer repeat the north and southward movement with the changing seasons. Most chum salmon return as adults at 3, 4, and 5 yr of age, with the 4-year-olds being the most abundant (Table 3). Asian chum salmon migrate farther east than North American fish move west. Tagging operations have shown that chums of Japanese origin travel east to near the coast of British Columbia (Neave et al. 1976). The Arctic coast chum salmon migrate south through the Bering Sea and overwinter in the Gulf of Alaska waters, and then migrate north again to the home rivers to spawn.

### *Chinook Salmon*

Chinook salmon are the largest (up to 45 kg) but least abundant of the five North American species of Pacific salmon (Figures 22 and 23). They typically spawn in the larger rivers and their tributaries, in relatively fast flowing water in beds of large gravel and stones. Chinook salmon are caught commercially along the coast from northern California to southeastern Alaska. Harvest rates from 1977 to 1986 were 2.6-4.2 million fish with the greatest catches occurring along the British Columbia coast (0.43-1.18 million, average 0.82 million) (Figure 22). Chinook salmon are also a valued sport fish and about 0.5 million are taken annually by anglers in North America.

*Ecotypes*. Chinook display a variety of life-history strategies (Table 3), including differences in age of seaward migration, variation in length of freshwater and ocean residence, distribution and ocean migratory patterns, and age and season of spawning migration. Two main behavioural forms can be recognized. "Stream-type" chinook salmon are typical of northern and of headwater tributaries of southern populations in North America. These fish spend one or more years in fresh water before migrating to sea, make extensive open ocean migrations, and return to the home stream in spring or summer several months before spawning. "Ocean-type" chinook salmon migrate to sea within three months of emerging from the gravel, spend most of their ocean life in coastal waters, return to the home stream in autumn, and spawn soon after arrival on the breeding grounds (Healey 1991).

Ocean-type chinook salmon occur only in spawning populations south of about 56°N (between the Taku and Sacramento rivers) and they remain close to shore in sheltered waters for several months. Stream-type chinook salmon occur more in northern spawning populations of northern British Columbia and Alaska, such as the Taku and Yukon rivers, and these fish distribute themselves farther into the Pacific Ocean (Healey 1991). All Asian chinook salmon are considered to be stream-type and therefore make extensive ocean migrations, whereas North American stocks consist of predominantly stream-type throughout Alaska and ocean-type south of the Alaska-British Columbia border. The exception are the chinook salmon from the Yakoun River on the Queen Charlotte Islands, which are stream-type (Healey 1991).

Stream-type chinook salmon spawn primarily in the larger rivers. In rivers with both stream and ocean-type chinook salmon, the former occupy the headwaters of the streams and the latter the lower reaches. The separation is not complete and the two forms do occur sympatrically on many spawning grounds (Healey 1991).

*Geographical Distribution.* The distribution of chinook salmon ranges from central California and northern Japan to rivers flowing into the northern Bering Sea (Figure 24D). On the Asian side they occur from northern Hokkaido to the Anadyr River. On the North American continent, chinook salmon are distributed from the San Joaquin River in California, northward to Kotzebue Sound in Alaska (Healey 1991). Chinook salmon have been introduced throughout the Great Lakes and in Missouri River mainstem reservoirs in the upper midwestern U.S. where they provide important sport fisheries. Chinook salmon are much more numerous on the North American side than on the Asian side of the North Pacific Ocean (Figure 22). In North America, the centre of distribution is near the Columbia and Fraser rivers (Healey 1991). In the south, the Sacramento River is an important chinook salmon stream. The largest commercial catches of chinook salmon occur in southern British Columbia waters (Figure 22).

*Age and Size.* Chinook salmon can reach maturity at a variety of ages (Table 3). The age of ocean-type stocks ranges from 2 to 6 yr (age 0.1-0.5) and of stream-type from 2 to 8 yr (age 1.0 - 2.5) (Healey 1991). Different river systems have different year classes and males generally mature earlier than females. In the Sacramento, Columbia, Fraser, Skeena, and Nass rivers most chinook salmon are 4 yr old. In the Yukon River, 6-year-old fish predominate. The younger ages (4 and 5 yr) contain more males and the older groups (7 and 8 yr) more females.

Newly emerged chinook salmon fry vary in size from 35 to 44 mm and weigh about 0.5 g. Growth rates can average from 0.20 to 0.33 mm/d in fresh water, to mean rates of 0.53 mm/d in southern and 0.84 mm/d in more northern estuaries. First year smolts can average in total length from 6.8 to 13.4 cm. Growth rates at this stage range from 0.077 to 0.17 mm/d when they are in or leave the estuary.

Chinook salmon are heaviest just before upstream migration and can reach a weight of 45 kg. In California, the lengths of 2, 3, 4, and 5 year-old chinook salmon are about 50, 70, 85, and 100 cm, respectively. Total mean lengths of chinook males of the Kamchatka River in the year of migration range from 58 - 70 cm for age 1.2 and from 73 to 89 cm for fish of age 1.3 (Grachev 1967). Chinook salmon lose 15-20% of body weight during migration to the spawning grounds and another 10-15% during spawning (Allen and Hassler 1986). Some male chinook salmon, especially from stream-resident populations, mature precociously without migrating to sea at the end of their first or second summer in fresh water (Healey 1991). In the McCloud River in California, Rich (1920) found that 10-12% of males matured early.

*Upstream Migration.* Many river systems have chinook salmon spawning runs that return at different (one to three) times during the year. Each run can be composed of several stocks, which spawn in particular parts of the river or in tributaries at a particular time of the year. The early appearing fish generally spawn farthest upstream in the headwaters and the later ones closer to the mouth of the river.

In southern areas chinook salmon runs tend to occur progressively later in the season, with early runs from May to July, and late ones from September to October. Some systems, such as the Fraser River, also have a small, third run in August. In the Columbia River the late August run is the largest, and the spring and summer runs are relatively small. The Sacramento River has spring and summer runs, as well a unique winter run, which returns at different times during the year (Healey 1991). Northern rivers, such as the Kamchatka, Yukon, Nass and Skeena rivers, typically have a single run, which may extend from April to August (Healey 1991).

*Reproduction.* Chinook salmon typically spawn in large river systems at several meters of depth, but will also spawn in small tributaries 2 to 3 m wide with only a few centimetres of water. They can spawn in significant numbers in areas from immediately above the tidal limit to locations over 1,000 km or more from sea as in the Columbia, Fraser, and Yukon rivers. Maximum depths of spawning range from 40 to more than 700 cm of water and the minimum depth is assumed to be 30 cm. Minimum velocities range from 10 to 52 cm/sec and maxima from 64 to 150 cm/sec (Healey 1991). Optimal gravel size for spawning ranges from 1.3 to 10.2 cm with 80% between 1.3 and 5.1 cm and 20% larger than 5.1 cm. Water velocities of 6 to 24 cm/sec are optimal for rearing juveniles and from 3 to 91 cm/sec for spawning (Allen and Hassler 1986).

Timing of spawning of chinook salmon varies greatly in different river systems. In the Sacramento River, the autumn run spawns from October to March, the winter run from May to July, and the spring run in September and October. Spawning is much more restricted in northern rivers and takes place in autumn. In the Fraser River tributaries spawning takes place from early July to late November and includes a summer and autumn run. In mainland British Columbia streams spawning occurs in August and September, in Vancouver Island streams in October, and in northern streams in September (Healey 1991). Spawning generally occurs at the lower end of pools, just above a riffle (Burner 1951; Briggs 1953). The spawning sequence and female-male interactions are as described in the section on Spawning Behaviour. Spawning times of females range from 5 to 14 days. After all eggs have been deposited females may defend the redd area against other females for another 4 to 26 d (Healey 1991).

Fertilized eggs are buried under 20-60 cm of gravel. Each redd contains several nests. Fertilization rate of eggs in natural conditions is very high (92% to over 99%) (Healey 1991). Chinook salmon have large eggs, ranging from 6.3 to 7.9 mm in diameter and weighing from 0.35 to 0.40 g (Allen and Hassler 1986). Fecundity of females varies greatly depending on size, age, and geographic distribution of fish. It can range from less than 2,000 to more than 17,000 eggs per female and generally increases from south to north. Average fecundity of females of the same size (74 cm) can vary from a low count of 4,347 to a high count of 9,427 eggs among different river systems from 41°N to about

60°N. This relationship may partly reflect a difference between stream- and ocean-type fish (Healey 1991). Most chinook salmon die after spawning, although some precocious males have been reported to survive (Healey 1991).

*Incubation and Emergence.* Incubation time of chinook eggs is dependent on temperature. Under constant temperature conditions the time from fertilization to emergence ranges from 236 d at 4°C to 96 d at 10°C (J. Jensen, Pacific Biological Station, Nanaimo, British Columbia, Canada, pers. comm.). Tolerance temperature limits during the embryonic life phase are from 5.8 to 14.2°C and the lower lethal temperature is 0.6°C and lower. Rate of survival during incubation at low temperatures is generally higher under changing ambient than under constant temperature conditions (Healey 1991). Although, survival of eggs in natural undisturbed redds can be high (82-97%), considerable mortality (up to 95%) can occur due to lethal temperatures, flooding, low dissolved oxygen, siltation, poor percolation, and oligocheate worm attacks (Briggs 1953; Vronskiy 1972).

Survival rates during incubation are poor at dissolved oxygen levels of less than 5 mg/l and intragravel flow rates less than 60 cm/hr (Briggs 1953). Also, high sediment levels during incubation are detrimental because they clog gravel interstices and reduce intragravel flow (Shelton and Pollock 1966). Emergence of fry usually takes place at night in spring from February to May. Emergence success is greatly affected by gravel size and can range from 13% in experimental troughs with small gravel sizes to 80-90% with coarse gravel (Shelton 1955).

*Juveniles.* After emergence chinook salmon fry generally disperse downstream. Initially they hide in the gravel and under banks during daylight hours, then appear along open shorelines and finally move into higher velocity waters along the shore or farther into the stream. During the first part of this process, they establish neutral buoyancy by filling the swim bladder, start feeding, and develop social behaviour (Healey 1991). In rivers farthest to the north most young chinook may stay in fresh water for at least a year (Healey 1991). In the southern parts of their distribution some chinook fry may move to salt water soon after emergence, whereas others may remain in fresh water from a few months to a year or more.

In the Nanaimo River chinook salmon occupy three geographically separated spawning areas (Healey and Jordan 1982). The fry from these three areas are genetically distinct and migrate seaward at different times. Fry from the lower spawning areas drift to the estuary soon after emergence, whereas fry from the middle area drift downstream at first and then rear in the river for 6-8 wk before migrating to sea. Fry of the upper spawning grounds spend a year in the river before their seaward migration as smolts. The latter hide under large rocks and debris during winter in fresh water. Upon smolting they change their behaviour and become less territorial, and migrate to sea in schools (Carl and Healey 1984). Downstream migration of chinook salmon smolts generally occurs during daylight hours in the upper 2 m of water. At night they disperse and swim deeper (Allen and Hassler 1986). Vertical diel movements increase as the season progresses and the juveniles move into deeper waters.

Fry migrating to sea generally remain in the estuary (maximum 60 d, average 20-25 d) and grow before moving on into the ocean at a size of 70 mm fork length. Yearling smolts migrating to sea may spend some time in the estuary or may disperse into the ocean directly after passing through the estuary (Healey 1991).

*Ocean Phase*. Chinook salmon yearling and fingerling migrants originating from streams north of northern Oregon move northward upon entering marine waters. Stocks from southern Oregon rivers travel primarily to the south (Healey 1991). The ocean distribution of chinook salmon in the North Pacific Ocean and Bering Sea extends from a southern range of 40°N in the west and 48°N in the east to 68°N in the north. Along the coast of North America, chinook salmon occur as far as 38°N (Healey 1991).

The movements of chinook salmon during their ocean residence are not well known. Stream-type chinook salmon seem to move offshore early and are the main type in open ocean waters. Ocean-type chinook salmon generally stay close to shore during the saltwater phase, although some may disperse far offshore. Chinook salmon tagged off southeastern Alaska were recaptured as far south as California and those tagged off California were recaptured as far as north as British Columbia. Fish tagged at intermediate points along the coast were recaptured both north and south of the release points (Healey 1991).

Chinook salmon generally occupy greater depths of water during their ocean residence time than other Pacific salmon. In west-coast Canadian waters, most immature fish occur at 20-37 m in the Strait of Georgia, at 48-55 m in Juan de Fuca Strait, and at depths of 40-60 m off the west coast of Vancouver Island. There is also an increase in average depth with time of season in Juan de Fuca Strait from 33 m in June and July to 41 m in August-October (Healey 1991).

In summer and autumn maturing chinook salmon return to their rivers of origin. Upon reaching the estuary, there may be a holding period before upstream migration or river ascent may take place almost immediately (Healey 1991).

### *Coho Salmon*

Coho salmon are the most widespread of the Pacific salmon species and are important in both commercial and sport fisheries. They are much more abundant in the eastern Pacific regions than in the west (Figure 22) and primarily use moderate-sized streams for spawning rather than large ones. Their breeding grounds seldom extend beyond 240 km above the river mouth. Coho salmon juveniles typically stay in fresh water for 1 year, and in more northern areas 2 yr, and then migrate to sea as smolts. They stay in the ocean for 18 months before returning to their natal streams to spawn.

*Ecotypes.* Two distinct types of coho salmon can be identified: An "ocean-type" that primarily occupies outer coastal or offshore waters, and an "inshore-type" that remains in near-shore waters during the saltwater life stage (Milne 1950). For coho salmon from the upper Columbia River to Alaska, Taylor and McPhail (1985) recognized a "coastal" form with large median fins and a deep robust body and an "interior" form with small median fins and a streamlined body. More or less distinct "summer" and "autumn" or "autumn" and "winter" runs of coho salmon have been described for certain Kamchatka stocks, but less definitely for North American stocks (Sandercock 1991).

Swain and Holtby (1989) noted differences in morphology and behaviour between juvenile coho salmon rearing in Mesachie Lake, British Columbia, and its tributary stream. The stream-reared juveniles are more aggressive than the lake-reared fish. Also the lake-type fish had shallower bodies and smaller, less brightly coloured fins. The authors concluded that the lower level of aggression, the more streamlined shape, and the reduced colouration of the lake-type juveniles appear to be adaptations to a schooling lifestyle in open lake waters. In some systems coho salmon may complete their life cycle in fresh water. This phenomenon occurs in natural systems, but in many cases the fish are forced to stay in fresh water as is the case with coho salmon that are transplanted to the Great Lakes (Sandercock 1991).

*Geographical Distribution.* The geographical distribution of coho salmon in Asia ranges from Peter the Great Bay and northern Hokkaido, along the coast of the Sea of Okhotsk, Sakhalin and the Kuril Islands, and along east and west Kamchatka to the Anadyr River. On the North American continent, coho salmon distribution extends from the Sacramento River in California north along the coast of Oregon, Washington, British Columbia, and southeastern, central, and western Alaska, through the Aleutian Islands to the Nome River and Kotzebue Sound in northern Alaska (Figure 24E). Coho salmon have also been introduced into the Great Lakes to provide sport fisheries.

Coho salmon are most abundant in streams from Washington to southeastern Alaska, with southern British Columbia as the centre of their distribution (Figure 22). In British Columbia coho salmon occur in 970 of the 1,500 documented salmon streams and are concentrated in the near coastal rivers and streams. Some, however, go as far as Shuswap Lake in the Fraser River and Babine Lake in the Skeena River system (Aro and Shepard 1967).

*Age and Size.* Coho salmon occur in 10 year classes (Table 3). Three (age 1.1) and 4-year-old (age 2.1) coho are the most common. These will have, respectively, spent one and two winters in fresh water and one winter in the ocean before returning to spawn. Mature coho salmon adults at the time of return to the stream range on average from 41 to 67 cm in length and weigh from 3.0 to 5.5 kg (Figure 23). The larger sizes

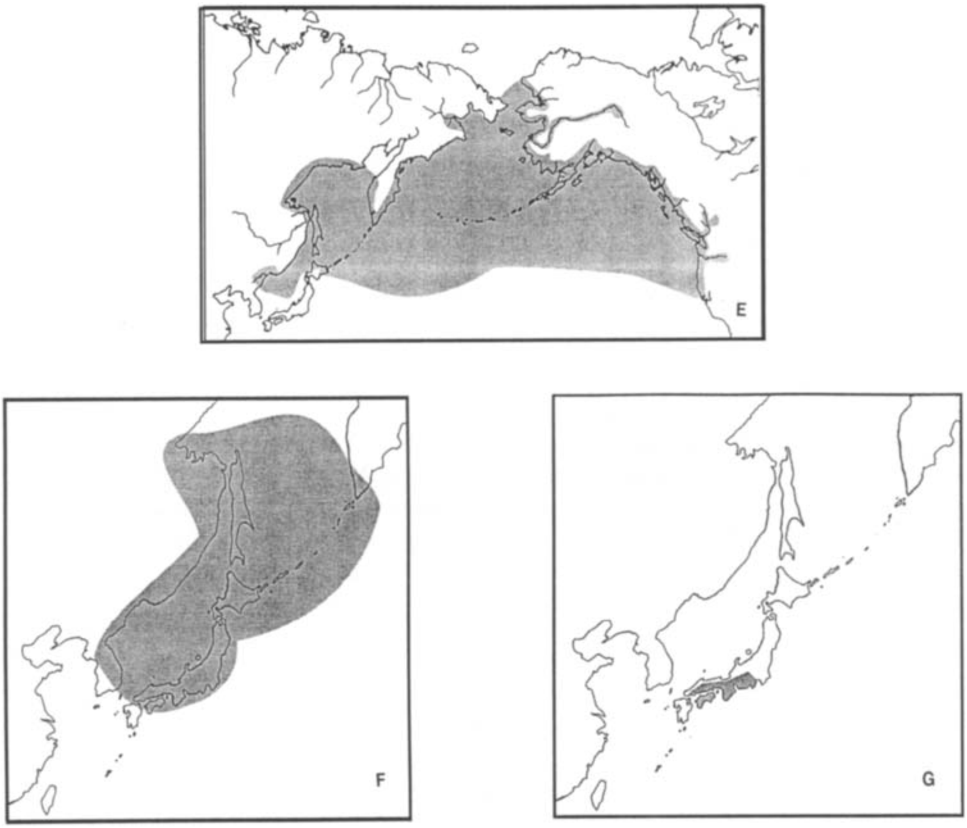

FIGURE 24 cont'd. Geographic distribution of (E) coho salmon, (F) masu salmon, and (G) amago salmon.

and weights are for 4-year-olds of age 2.1 and 1.2. Little difference in weight is evident between males and females. The largest coho salmon on record weighed 14.0 kg and was caught off Victoria, British Columbia (Hart 1973). Catches of 6-kg coho salmon are not unusual, but fish larger than 9.0 kg are rare (Sandercock 1991). Maximum sizes in California are 13.6 kg in weight and 80 cm in length (Hassler 1987). Coho salmon that return as jacks after only a few months in salt water average from 27 to 36 cm in British Columbia and up to 41 cm in California (Sandercock 1991).

Upon emergence, coho salmon fry are about 30 mm long. After a month in the stream they reach a length of 52 mm. Smolts migrating downstream to sea are relatively consistent in length over the geographical range and average from 10 to 12 cm in fork length with mean weights of 11-18 g. Two-year-old smolts can be between 11 and 15 cm long, with weights ranging from 15 to 34 g (average 25 g) (Sandercock 1991).

*Upstream Migration.* In the western North Pacific Ocean coho salmon appear in coastal waters mainly during late July and August. Before entering the home streams, they may hold for some time in bays or estuaries. River ascent can be very prolonged and takes place mainly during daylight hours. Stream entry often coincides with rising water levels in autumn due to heavy precipitation and at water temperatures between 4 and 10°C. In many of the coastal streams in which coho salmon spawn, upstream migration is short. In large rivers migrations are generally not farther than 240 km. However, coho salmon can perform longer migrations in the very large rivers. For example, in the Skeena and Fraser river systems coho salmon will travel to over 600 km upstream and in the Yukon and Porcupine rivers, distances of 1,800 and 2,200 km, respectively, have been recorded (McPhail and Lindsey 1970; Sandercock 1991).

*Reproduction.* Coho salmon choose a greater variety of stream types for spawning and spawn over a longer time period than other Pacific salmon. Spawning occurs in numerous small coastal streams, in large rivers, and in remote tributaries, and can last from October to March. Most populations, however, spawn between November and January. Spawning can take place at a wide range of temperatures, ranging from 0.8-7.7°C in Kamchatka, to 5.6-13.3°C in California. In general spawning temperatures range between 6 and 12°C. Depth of water levels on spawning grounds ranges from 10 to 45 cm and water velocities vary from 18 to 76 cm/sec (Hassler 1987). Acceptable gravel substrate-size ranges are 1.3-10.2 cm, 3.8-12.7 cm, and 7.5-15.0 cm for different coho salmon-spawning streams (Sandercock 1991).

Coho salmon are aggressive fish and defend much larger nesting territories than other Pacific salmon. They require an inter-redd space that is at least three times the redd size or an area of about 12 $m^2$. Nests are prepared by the female in areas with ground water seepage at the head of a riffle (Sandercock 1991). Their spawning behaviour is very similar to that described in the section on Spawning Behaviour.

Redd sizes range from 115 to 195 cm in length and from 100 to 135 cm in width, and on average cover an area of 2.6-2.8 $m^2$. Eggs are buried at depths ranging from 18 to 39 cm and a redd can contain four to five egg pockets (Sandercock 1991). Fecundity of coho salmon females ranges from 2,000 to 5,000 eggs per female. Fecundity increases from California to Alaska and is higher in North America than in Asia. Average egg diameter is 4.5-6 mm, which is smaller than for most other Pacific salmon (Sandercock 1991).

*Incubation and Emergence.* The optimum temperature for coho salmon egg incubation is between 4 and 11°C. However, in Kamchatka temperatures during incubation may range from 0 to 3.5°C (average 2.2°C). At these temperatures hatching occurs after 137 days. The generally higher temperatures in North American streams result in shorter incubation periods. In the Big Qualicum River, Fraser et al. (1983) found that the total heat requirement from egg fertilization to emergence was 1,036 to 1,380 degree-days of accumulated temperature units. J. Jensen (Pacific Biological Station, Nanaimo, B.C., Canada, pers. comm.) determined that under laboratory conditions, the time needed from egg until emergence for coho salmon was 180 d at 4°C, 118 d at 7°C, and 81 d at 10°C. The incubation period of coho salmon eggs is longer at dissolved oxygen levels of 5 mg/l or less. After hatching, the alevins move down into the gravel and then hold for several weeks. During this time the young absorb most of the yolk sac and when the time for emergence approaches, they move up to the gravel-water interface and emerge into the stream as fry (Dill 1969). Emergence generally occurs from early March to late July and takes place primarily at night.

*Juveniles*. Upon emergence coho salmon fry move to shallow gravel areas near the stream banks, not far from their redds, and tend to aggregate in groups, feed actively, and grow rapidly. As the fry grow larger, they leave the aggregations and disperse both upstream and downstream. They also become aggressive and territorial and defend certain areas in the stream against conspecifics. Hierarchies and territories are generally organized on the basis of fish size. The smaller fish are continuously harassed by the larger "residuals" and become "nomads" (Chapman 1962).

In summer, juvenile coho salmon live near the stream surface and prefer a combination of pools and riffles with little sediment. They occupy streams that are protected by large woody debris and that have undercut banks and overhanging vegetation. They prefer water temperatures ranging from 10 to 15°C and dissolved oxygen levels that are near saturation. In winter, the juveniles move to mainstream and backwater areas and to pools in secondary channels with vegetation cover and undercut banks. Some coho salmon fry rear in estuaries in salinities ranging from 8 to 25 ‰ and grow faster under such conditions than fry in fresh water.

Almost all young coho salmon spend a year or more in streams or lakes before they migrate to sea. As they grow in fresh water, they move into deeper waters and eat coarser food. During the winter months feeding declines and growth becomes negligible. Downstream migration of coho salmon smolts towards salt water takes place in spring and summer, mainly from April to August. The timing varies between streams and annual variations are also associated with stream conditions. The impetus for migration of the smolts appears to be related to size, age, water level, and stream discharge. The larger fish migrate first and the main migration occurs at night (Sandercock 1991).

*Ocean Phase*. After reaching salt water, juvenile coho salmon remain in shallow coastal waters for a while. By late July they start to move off shore into deeper waters. This movement lasts until about mid-September. Some coho salmon migrate far into the North Pacific Ocean (ocean-type) and many remain in coastal waters (inshore-type) (Milne 1950). The ocean type grows faster than the inshore type. This faster growth begins in fresh water and continues through the marine phase.

The main mass of Asian and North American coho salmon is distributed farther to the south in the North Pacific Ocean during spring and early summer than other

Pacific salmon. Ocean residence time of coho salmon is generally 9 to 10 months, that is, they spend only one winter at sea before returning to their natal stream. Coho salmon that return to the east coast of Kamchatka and more northern areas approach coastal regions over a broad front. Some enter the Bering Sea first through the western Aleutian passes and then turn west or northwest toward the coast of Asia. On the North American side, coho salmon arrive in coastal waters early in July and runs may extend from late August to September. Northern stocks generally appear earlier than southern populations. Coho salmon may hold for a while in bays and estuaries before migrating upstream. Homing appears quite accurate with straying rates of around 0.1% (Sandercock 1991).

### *Masu Salmon*

Masu salmon only occur on the Asian side of the Pacific Rim countries. They can be separated into an anadromous form, called sakuramasu (*Oncorhynchus masou masou*), and a non-anadromous or freshwater form, called yamame or yamabe (*O. m. ishikawae*). Sakuramasu means "cherry trout" in Japanese because the return of these fish to their natal streams coincides with the appearance of the cherry blossoms in Japan from March until May (Kato 1991). The anadromous fish die after spawning, whereas yamame males may return for spawning several times (Kato 1991). The name masu will refer to both forms in this section unless mentioned otherwise.

*Ecotypes*. The main two ecotypes of masu salmon are the anadromous and non-anadromous forms. The anadromous form only has a few variations on the common life cycle pattern of one to two winters in fresh water and one to two winters in the ocean (Table 3). In northern areas, both periods of freshwater and saltwater residence can be one year longer, with the oldest fish recorded as age 3.3 (Birman 1972). Some masu, especially the males, can be non-anadromous in southern areas and spawn at age 1.0. All masu returning from the sea are considered to die after spawning. However, male yamame are known to spawn several times (Machidori and Kato 1984).

*Geographical Distribution*. Masu salmon occur almost all through Japan and along the eastern Siberian coast, between latitudes 36°N and 55°N. A small relict occurs in one lake in Taiwan (Figure 24F). They have a more southerly geographic distribution than the other species of Pacific salmon, except for amago salmon. The rivers in which masu salmon spawn are primarily in the region of the Sea of Japan and Sea of Okhotsk. In Japan they occur on Hokkaido, Honshu, and the northern part of Kyushu. Some rivers on the Pacific Ocean side of Hokkaido and Honshu also contain spawning populations of masu salmon. On the mainland coast of Asia masu salmon are distributed from northern Korea along the Primore coast to the Amur River in the north, and along the west coast of Kamchatka from the Bolshaya to the Icha River. Masu spawn in many rivers on Sakhalin Island, especially the southern part, and Kunashir and Iturup islands of the Kuril Islands chain (Kato 1991).

The geographic distribution of freshwater masu, or yamame, extends farther south than for the anadromous form. It includes all of Japan, except for the southern part of Shikoku. Yamame also occur in Taiwan in some streams in the Formosan highland area and in some streams in western South Korea (Figure 24F) (Kato 1991).

*Age and Size.* Masu salmon fry leaving the gravel are about 3-3.5 cm in fork length. By July they have grown to 7-8 cm and by September to 9-11 cm (Kato 1991). By late autumn the anadromous juveniles are 10-13 cm long and turn slightly silver. The stream-type are from 7.5-11 cm at about that time, with some males showing signs of maturing.

Masu salmon are difficult to age. Japanese fisheries biologists consider that this species mainly matures when 3 to 4 yr old or at age 1.1 and 2.1, whereas their Russian colleagues believe that maturity occurs primarily when they are 4 to 5 (1.2 to 2.2) or 5 to 6 yr (2.2 to 2.3) of age (Machidori and Kato 1984).

Size and weight of spawners varies from region to region, ranging from 35 cm fork length and 0.3-0.4 kg in weight to more than 70 cm in length and 5 kg in weight. Masu salmon from Honshu average 56-58 cm in length and 2.6 kg in weight, and from Primore 54-66 cm in length and 2.3-4.8 kg in weight. These fish are generally larger than those from Sakhalin (47-54 cm, 1.9-2.2 kg) or Hokkaido (40-53 cm, 0.9-1.9 kg) (Kato 1991). Yamame are only half as large as anadromous masu. They grow poorly under freshwater conditions and may only average 20 cm in length 2 yr after hatching and 25 cm after 3 yr (Kato 1991).

*Upstream Migration.* Maturing masu salmon migrate to coastal waters early in the year and start their upstream migration soon after snow melt. Upstream movements peak from late spring to early summer in some systems and from late summer to early autumn in others. River ascent occurs progressively later from south to north over the distribution range of masu. The duration of upstream migration varies for different regions and generally lasts from two to three months. The early groups usually migrate to the upper reaches of the streams and the late groups, which enter the rivers just before spawning, spawn in the middle and lower reaches of the stream. Upstream migration takes place throughout most of the day but is more intense during dusk and dawn. Migration is also influenced by river discharge (Kato 1991). Upstream migration occurs in water temperatures ranging from 6 to 17°C; optimum temperatures are from 9.5 to 15.4°C in June and July. Upon entering the home river, masu usually hide in deep pools in the main streams or in large tributaries. As spawning time approaches in autumn, the mature adults move to upper parts of streams or to tributaries and choose shallow areas with swift currents and with clear water (Kato 1991).

*Reproduction.* Masu salmon spawn in both small streams and large rivers. Spawning begins as early as the end of July in the north and as late as early October in the south. It reaches its peak from August to September and on average lasts from 30 to 40 d in each river. Movement from deeper waters to the spawning grounds generally occurs when water temperatures exceed 12°C. In some rivers, masu may enter late in the season and start spawning soon after entry. Spawning can occur in water temperatures ranging from 7 to 20°C, but mainly takes place between 11 and 15°C (Kato 1991).

The spawning behaviour of masu is similar to that described above in the section on Spawning Behaviour. Females select nest sites in shallow and fast flowing waters at the lower ends of pools or along the river banks. Current speeds on the breeding grounds range from 12 to 101 cm/sec and average 46-56 cm/sec. Finished redds are from 95 to 410 cm long (average 124-210 cm) and from 45 to 165 cm wide (average 74-84 cm) (Kato 1991). The gravel composition of spawning areas can range from 7 to larger than 61 mm in size. Spawning streams usually have a dissolved oxygen content of about 8-10 mg/l and a pH of 6.8-7.2 (Kato 1991). Females are often attended by two or three anadromous and several stream-type males. Nest digging activity starts early in the morning between 0400 and 1000 h. Sometimes, spawning activities can be completed by late afternoon of the same day that nest building started, but usually it continues the next day. Depths of nests range from 3.5 to 64 cm and average from 12 to 45 cm (Kato 1991). Average time for nest building and spawning is 10-23 h and thereafter the female protects the redd area for 2 to 8 days. The anadromous and non-anadromous forms of masu have been observed to spawn together. Especially, yamame males can mate with masu females. Most masu die after spawning, however, yamame males may spawn two or more times.

Fecundity of masu salmon females is related to size. The larger females of the Honshu area have 3,000-3,850 eggs (average 3,400). On Hokkaido fecundity is 3,000 in the western, 2,600 in the southern, 2,100 in the eastern, and 1,900 eggs in the northern areas (Kato 1991). In southern Sakhalin and the Kuril Islands, masu females are relatively small and have about 1,600-1,700 eggs. The diameter of masu salmon eggs ranges from 4.5 to 7.7 mm with an average of about 6.0 mm. Larger females tend to have larger egg sizes. Yamame females have fecundities averaging from 200 to 300 eggs, with an egg diameter of about 5 mm.

*Incubation and Emergence*. The eggs of masu incubate in the gravel during the late autumn and winter months. Development of embryos and the emergence of fry are closely related to temperatures. At a constant temperature of 8°C hatching occurs after 55-60 days. Under ambient temperature conditions in a stream, development may take up to three months. In total, masu eggs require 430-480 temperature units (above 0°C) to time of hatching. Yamame eggs hatch 85-90 d after fertilization at 6°C, about 50 d at 10°C, and 35 d at about 14°C (Kato 1991). Survival from fertilization to hatching is highest and abnormality rates lowest, when eggs are incubated at temperatures between 7 and 11°C (Kato 1991). In Hokkaido, the alevins of masu salmon hatch from November to December and the fry emerge from the gravel between late March and early May.

*Juveniles and Immatures*. After leaving the gravel beds, masu fry spend some time in dense schools in shallow backwaters near the spawning grounds. As they grow through the summer they start to disperse and take up residence in deep pools. Here, they stay for an extended period of time and appear to defend feeding territories. Territory defence is most intense for several hours after sunset (Kato 1991).

When water temperatures begin to drop and precipitation increases in late September or October, juvenile masu move to warmer water areas downstream or to spring-fed tributaries. During this time the sea-run types become slender and their body surface begins to turn slightly silver or white in colour. The stream-type fish, on the other hand, increase their body depth, retain their parr-characteristics, and are generally sturdier. Some males may start to show signs of maturing. During the winter, the juveniles hide in the deeper parts of the streams near banks covered with bamboo or dead grass (Kato 1991).

Anadromous masu salmon change into smolts after one winter in fresh water in the southern parts of their geographic distribution and after two winters in more northern regions. Migration to sea takes place during spring and early summer from late April to early June and occurs earlier in the season in southern and later in northern areas. Smolt migration is most intense for several hours after sunset (Kato 1991). There is a south-north geographical shift in the sex ratio of masu smolts ranging from three to four times as many females as males in the south to equal proportions of both sexes in the north. This is because in southern areas most males are non-anadromous and tend to mature early, whereas in northern areas most migrate to sea (Kato 1991).

*Ocean Phase*. When the smolts of masu salmon have entered salt water by late April they start to school. Some may stay in coastal waters, whereas others become more active and start their ocean migration. By July most of the juveniles have left the coastal areas and have moved north, probably to the Sea of Okhotsk, where they stay during the summer. Masu grow rapidly during the summer and greatly increase in size. As water temperatures become colder in autumn the immature fish migrate south to southern and southwestern parts of the Sea of Japan and concentrate in the vicinity of 38°N and northward along the island of Honshu. The fish that stay in the ocean for one or more years move north again by April towards the Sea of Okhotsk, whereas the maturing adults return to their home river. In general, masu return to their natal stream for spawning. However, tagging experiments indicate that some straying does occur (Kato 1991).

### *Amago Salmon*

Of the seven species of Pacific salmon, least is known about the life history of amago salmon. This species generally has a complete freshwater life cycle and, uncharacteristically for Pacific salmon, not all fish die after spawning. Especially males may reproduce two or more times.

*Ecotypes*. Amago salmon exist in two ecotypes, i.e., a stream type, *Oncorhynchus rhodurus* var. *rhodurus*, called amago salmon, and a lake-dwelling type, *O. r.* var. *macrostomus*, called biwamasu. These two types are different in morphology and ecology. The stream-type amago remains in rivers during its life cycle, and some, primarily females, may migrate to sea (Kato 1991). The lake-dwelling biwamasu originally only occurred in Lake Biwa but has been transplanted to other lakes in Japan. The name amago will be used for the stream-type form and biwamasu for the lake-dwelling form (Kato 1991).

*Geographical Distribution*. Amago salmon occur naturally only in southern Japan (Figure 24G). They range from the northern tip of Kyushu northward throughout Shikoku and along the Pacific Ocean side of Honshu as far as Tokyo Bay. Their ability to tolerate warmer water than the other Pacific salmon species is reflected in their more southern distribution. Stream-type amago are found in southwestern Honshu, Shikoku, and the northern tip of Kyushu (Kato 1991). The lake-dwelling biwamasu occurs naturally only in Lake Biwa and its northern tributaries. Transplants of biwamasu have been made to Lake Chunzji and Lake Ashinoko (Masuda et al. 1984).

*Age and Size*. Stream-type amago generally mature at age 1.0 and 2.0 as males and at age 2.0 as females. Biwamasu males mature at age 2.0 to 4.0 and females at age 3.0 to 4.0 (Figure 19) (Kato 1991). Biwamasu usually mature at a later age and are larger at the time of spawning than amago salmon. In the Ado River, the lengths of biwamasu range from 22 to 24 cm and the amago salmon are 12 to 18 cm long. Sea-run amago may grow to more than 50 cm long when mature (Kato 1991).

*Upstream Migration*. Stream-type amago start migrating upstream for spawning between early April and June, with a peak in May. Water temperatures at the start of migration are 10-12°C (Kato 1991). Lake-dwelling biwamasu in Lake Biwa inhabit waters below 20 m in summer, stay near the surface in winter, and occur at all depths during the rest of the year. In general, they prefer water of about 13°C. Migration to the spawning tributaries of the lake takes place between September and November (Kato 1991).

*Reproduction*. After entering the natal stream in spring and summer, the maturing adults of amago hold in the deeper parts of the river until they move to the breeding grounds in autumn. Spawning generally occurs from October to December. Biwamasu spawn in the lower or middle reaches of rivers. Spawning occurs at temperatures of about 10°C (Kato 1991). The behaviour activities of amago during spawning are similar to other Pacific salmon. Nesting territories of amago salmon are about 1 $m^2$ in area. Water depths in nesting areas range from 10 to 30 cm (average 22 cm ) and average current speeds range from 0 to 30 cm/sec. Redds contain up to three batches of eggs and are about 53 cm long and 43 cm wide.

Amago salmon eggs are light yellow in colour and vary from 4 to 6 mm in diameter. Females 12-18 cm long have 75-209 eggs (average 150). Sea-run females can have between 1,000 and 2,500 eggs (Kato 1991). Fecundity of biwamasu females ranges from 800 to 3,000 eggs per female. Ado River biwamasu 22-24 cm long have from 428 to 815 eggs per female (Kato 1991).

*Incubation and Emergence.* Amago salmon eggs hatch out after 30-50 d at 9-16°C. The alevins then stay another 30 d in the gravel until the yolk sac is absorbed. Emergence of the fry occurs in early spring (Kato 1991).

*Juveniles, Immatures and Adults.* The fry of amago salmon stay in the spawning river after emergence from the gravel in spring. They grow rapidly after they start to feed and swim freely. In summer the young amago spread out into tributaries with water temperatures lower than 20°C. In winter, they move to upstream or midstream areas or, in the case of biwamasu, into lakes (Kato 1991). Little is known about the behaviour of biwamasu until they reach maturity and return to the spawning grounds in the river during autumn.

Some anadromous juvenile amago start to smolt by the end of October and migrate to salt water in December. The smolts usually stay in the river mouth for one month and then disperse through the estuary or bay. More than twice as many female smolts as males migrate from the Nagara River to sea in January and almost up to 3.5 times as many females as males migrate from January to April (Kato 1991).

*Ocean Phase.* Ocean distribution is limited to bays and inlets and only a few will migrate farther off shore (Machidori and Kato 1984). After about 4 to 6 months in the sea, the maturing fish gather in estuarine waters during April and May and quickly migrate upstream from mid-April to May. Tagging studies have shown that 95% return to their river of origin (Kato 1991).

## THE PACIFIC TROUTS

The native trouts of western North America include the rainbow and cutthroat trout. These two species were originally taxonomically linked with the Atlantic or Eurasian trouts of the genus *Salmo*. However, on the basis of evolutionary evidence presented by Smith and Stearley (1989; *see also* Stearley and Smith 1993), which showed that rainbow and cutthroat trout have a greater genetic affinity to the Pacific salmon than to the Eurasian trouts, they were renamed *Oncorhynchus mykiss* and *O. clarki* (Behnke 1992). In this chapter these two species are referred to as Pacific trouts.

The life cycles of rainbow and cutthroat trout are similar in many ways to those of other salmonids, except that they spawn in spring instead of autumn. Pacific trout fry, however, still emerge from the gravel during late spring or early summer shortly after the Pacific salmon fry, during the time of spring bloom. In streams where Pacific trout and

Pacific salmon occur sympatrically, the juveniles of the latter have a head start because their embryonic development commenced in autumn, several months earlier. This often leads to a disadvantage in competitive situations for food and space. However, because of rapid growth of the Pacific trout young, they quickly overcome the size advantage of the Pacific salmon juveniles. In many cases the young of the two groups are not in direct competition because they occupy different parts of riverine and lake habitats.

### *Rainbow Trout*

The rainbow trout is endemic to western North America and eastern Asia and has non-anadromous and anadromous forms. The non-anadromous form occupies a wide variety of freshwater habitats and occurs in stream-resident and lake-resident forms. Anadromous rainbow trout, called steelhead, migrate to sea as juveniles and can travel great distances in the ocean. The steelhead from Asia and North America were once considered different species but are now recognized as one (Behnke 1992).

*Ecotypes*. The rainbow trout exists in a number of distinctive ecological forms and subforms, which differ in morphology, behaviour, and life history traits. An important ecological separation exists between non-anadromous and anadromous forms. Non-anadromous rainbow occur in a great number of ecotypes of which the more important ones are the coastal rainbow, Columbia River redband, California golden, Kern and little Kern golden, Sacramento redband, and the Kamchatkan rainbow trout (Behnke 1992). Some authors also include the Gila and Apache trout in this group (Scott and Crossman 1973). Behnke (1992) recognized the last two trout as belonging to a separate species, *Oncorhynchus gilae*. Others recognize golden, Gila and Apache trout as three distinct species (*see* Chapter 2, Table 1).

Steelhead, the anadromous form of rainbow trout, have a complex life history with regional differences and varying time periods in fresh and salt water. In North America, steelhead are separated by the Cascade Mountains into a coastal and an inland group, which are genetically distinct from each other (Allendorf 1975; Parkinson et al. 1984; Okazaki 1984). In addition, steelhead occur in two behavioural forms, which have a marked variation in their time of river entry and upstream migration. Stocks that return to the home river during autumn and winter (December through March) are classed as "winter" steelhead, whereas those that enter and ascend streams during spring and summer (May through August) are named "summer" steelhead (Withler 1966; Smith 1968).

California stocks are primarily winter-run steelhead, whereas Idaho fish are entirely summer-run. The coastal stocks of Washington, Oregon, and British Columbia are a mixture of both winter- and summer-run steelhead with the winter-run making up the greater portion. The inland populations of the Fraser and Columbia river systems and of central Alaska westward to the Alaska Peninsula and in Asia are primarily composed of summer-run steelhead (Burgner et al. 1992). A special type of steelhead is found in river systems in southern Oregon and northern California in the Rogue, Klamath, Mad, and Eel rivers (Barnhart 1986). These steelhead return to fresh water after only a few months in salt water, much the same as coastal cutthroat trout (Burgner et al. 1992) They grow to an average weight of about a half pound (230 g) during their short ocean residence time and are called "half-pounders".

*Geographical Distribution.* The natural geographical distribution of rainbow trout includes freshwater systems along the eastern Pacific Ocean, west of the Rocky Mountains, from the Rio del Presidio River in Mexico to the Kuskokwim River in southwestern Alaska (Figure 25A) (Behnke 1992). They also occur naturally in the drainages of the Peace and Athabaska rivers east of the Rocky Mountains (Nelson and Paetz 1992). In Asia, rainbow trout are most abundant on the Kamchatka Peninsula. They are also found in streams on the west coast of the Sea of Okhotsk and in streams of Greater Shantar and Komandorskiy islands (Savvaitova et al. 1973).

The distribution of steelhead ranges from the Sacramento and Klamath rivers in northern California, north through Oregon, Washington, British Columbia, and southeastern, central, and southwestern Alaska, to the south side of the Alaska Peninsula and Umiak Island (Figure 25B) (Burgner et al. 1992). The Columbia River Basin is the centre of abundance in North America and produces 29% of the total coastwide population; approximately 14% originates from British Columbia streams (Light 1987).

Rainbow trout have been introduced widely outside their natural range throughout North America and other parts of the world, such as South America, Europe, southern Asia, Japan, Africa, Australia, and New Zealand (MacCrimmon 1971). The trout that were introduced into the Great Lakes were of steelhead origin and have partially retained their original life history pattern, using the large lakes as they would an ocean environment (Scott and Crossman 1973).

*Age and Size.* The life span for southern populations of rainbow trout averages 3-5 years for many stream and lake populations. Female rainbow trout become sexually mature during their third year and males during their second or third year (Raleigh et al. 1984). Some females can reach sexual maturity as late as 6-year-olds and some males as early as 1-year-olds (Scott and Crossman 1973). Maximum size of rainbow trout varies with stock, region, and habitat. They can grow to 122 cm long and 16 kg in weight, but the average is 3.6-4 kg.

The life expectancy for steelhead is 4-8 yr and the average size of fish returning to fresh water is between 62.5 and 75 cm. Large sizes are attained by summer fish of the Clearwater River, Idaho (83 cm), and the Kispiox (85 cm) and Thompson (91 cm) rivers in British Columbia (Burgner et al. 1992).

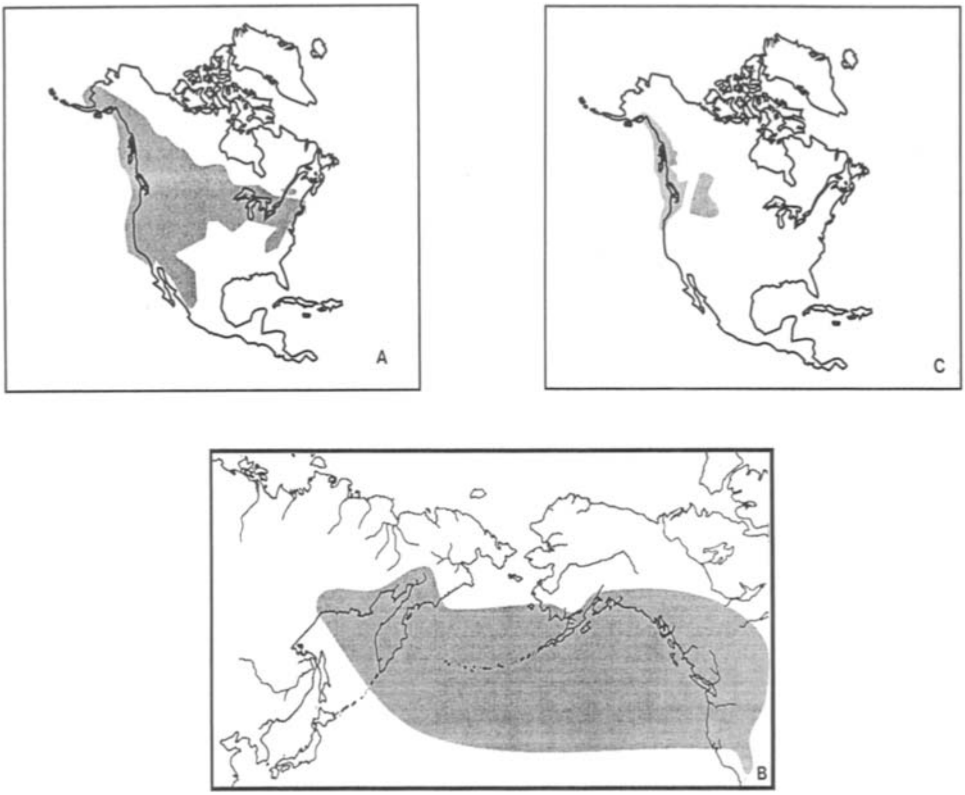

FIGURE 25. Geographic distribution of (A) rainbow trout, (B) steelhead trout, and (C) cutthroat trout.

*Reproduction*. The time of spawning of rainbow trout ranges from January to July (mainly from mid-April to June) depending on location. Spawning usually commences one month earlier in outlet than in inlet streams of lakes (Lindsey et al. 1959). Although summer-run steelhead enter streams in spring and early summer and winter-run fish in autumn and winter, both groups spawn in spring and early summer from March to early July. Spawning occurs in many small streams and in suitable portions of main streams, such as the Columbia, Fraser, and Skeena rivers. Summer steelhead select smaller streams than winter steelhead. The streams selected for spawning are cool, clear, and well-oxygenated, between 10 and 150 cm deep, and with current velocities of 23-155 cm/sec (Reiser and Bjornn 1979; Sheppard 1972). Water temperatures range from 4 to 13°C with a peak at 8°C (Reiser and Bjornn 1979). Optimal gravel size is 1.5-6 cm for spawners smaller than 50 cm and 1.5-10 cm for females larger than 50 cm (Orcutt et al. 1968).

Spawning behaviour of rainbow trout and steelhead is typical for salmonids (*see* section on Spawning Behaviour). Nest building continues day and night and generally the female digs several nests (two to five) in succession, laying about 800-1,000 eggs in each nest pocket. After the female is spent the male leaves her. She continues to stay over the redd site to further shape the gravel mound and to defend the area against other females. Female steelhead do not guard their redds after spawning and tend to leave the spawning area by passively moving downstream as soon as all the eggs have been laid (Burgner et al. 1992). Not all rainbow trout die after spawning. Typical survival rates of repeat spawners is between 10-30%, but can range from extremes of 1% to more than 65% (Raleigh et al. 1984). The trend toward repeat spawning increases from north to south (Withler 1966).

The fecundity of rainbow trout is highly variable and is related to size. Minimum and maximum fecundity ranges from 200 to 12,700, but generally is from 500 to 3,200 eggs for stream-resident and from 900 to 4,600 eggs (average about 2,000) for lake-resident females. The eggs are 3-5 mm in diameter and pink to orange in colour (Scott and Crossman 1973; Raleigh et al. 1984).

*Incubation and Emergence.* In contrast to most other salmonids (except cutthroat trout), rainbow and steelhead trout eggs develop in regimes of rising temperatures. The number of degree-days for development of steelhead eggs to emergence is much less than for Pacific salmon (Brannon 1987). Incubation times vary from 28 to 49 d until hatching and from 45 to 75 d until emergence, depending on temperatures (Raleigh et al. 1984). Optimal temperatures for incubation are between 7 and 12°C. Dissolved oxygen levels required for incubation need to be greater than 7 mg/l for temperatures below 15°C. Rainbow and steelhead trout fry remain in the gravel for about 2 to 3 wk after hatching before emerging from the gravel at night. The fry emerge from April to May when spawning is in early spring and from mid-June to mid-August when spawning has occurred in April or May. The timing of emergence varies with water temperature.

*Juveniles and Immatures.* After emergence, fry of stream-resident fish initially stay in shallow water close to the banks and then leave the schools to move into deeper parts of the stream to establish territories among rocks and in riffle areas (Raleigh et al. 1984). Fry of lake-resident rainbows may move up or down the spawning river almost immediately after emergence, or may overwinter in the spawning stream during their first growing season and enter a lake the following season.

Young rainbow trout generally occupy shallow water areas that have cover provided by vegetation, debris, and rocks. Preferred stream velocities are less than 8 cm/sec. As they grow older they tend to move to deeper and faster flowing water with velocities between 10 and 12 cm/sec to a maximum of 22 cm/sec (Raleigh et al. 1984). Optimal temperatures for juvenile rainbow trout are between 15 and 20°C. Steelhead require temperatures between 7 and 10°C (range 5-12°C), especially from March until June, for normal smoltification to occur (Raleigh et al. 1984).

Juvenile steelhead may remain in fresh water from 1 to 5 yr, usually 2 yr, before smolting. The onset of smoltification depends on size and occurs when the fish have reached a length of 15-20 cm. Steelhead can spend one to three winters in salt water and grow rapidly in the ocean. Most return to the home river after two (64%) or three (32%) winters in the sea. Males generally return at an earlier ocean age (at .2 ocean age) than females (at .3 ocean age) (Ward and Slaney 1988). The length of residence in both fresh and salt water increases from south to north (Withler 1966).

*Movements and Migration.* Fry of lake-resident rainbow trout populations migrate to the nursery lake from inlet and outlet spawning grounds soon after emergence. Little is known about movements in the lake until the mature adults return to the spawning grounds. Rainbow trout start to move to the spawning grounds in winter and early spring, in some cases while ice is still on the lake. Stream-resident juveniles may stay in the tributary stream of birth during their whole life cycle or may move to the mainstem of the river when water conditions in their natal stream become unacceptable. They move to the spawning areas in early spring.

Steelhead juveniles usually stay in streams for 2 yr (range 1-5 yr) before they migrate to sea as smolts. Smolt migration takes place from mid-March to mid-July with a peak between mid-April and mid-May at the time of spring run-off. In the Columbia and Fraser River systems smolts migrate in June (Withler 1966). Soon after ocean entry, the young steelhead move quickly offshore and can travel thousands of kilometres into the Pacific Ocean within a few months (Hartt and Dell 1986).

The distribution of steelhead in the North Pacific Ocean is limited by surface-water temperature boundaries of 5°C in the north and of 15°C in the south (Burgner et al. 1992). Within this area, ocean migration tends to be north and eastward from autumn through winter and south and westward from spring through summer. Summer-run steelhead enter streams in spring and early summer and winter-run fish in autumn and winter. Because both groups spawn in spring and early summer, the summer steelhead arrive at the natal stream 9-12 months and winter fish 3-4 months before spawning. The summer-run fish have only slightly developed gonads at the time of stream entry, whereas gonads of winter steelhead can be in various stages of development (Burgner et al. 1992).

Upon returning to the home stream steelhead generally do not linger in the estuary but enter the stream immediately if flows are adequate (Burgner et al. 1992). Upstream migration is facilitated by freshets and proceeds best when streamflow conditions are above normal seasonal flows and temperatures are above 4°C and below 18°C (Withler 1966; Raleigh et al. 1984). Most steelhead do not feed when they have returned to fresh water. As in many of the salmonids, rainbow and steelhead trout show a high degree of homing when they return to their natal stream for spawning. In Loon Lake, British Columbia, 94% of the rainbow trout that were mixed when in the lake, separated into inlet and outlet spawning groups when they returned to their ancestral breeding grounds (Scott and Crossman 1973). In Waddel and Scott creeks, Shapavalov and Taft (1954) reported homing rates of 98.1% and 97.1%, respectively.

Half-pounders migrate to the ocean during spring and early summer as smolts and return to fresh water from August to early October after a short period at sea. In contrast to steelhead, they feed extensively in fresh water. The surviving fish return to the ocean the following spring and migrate back to fresh water as mature steelhead in summer and autumn (Barnhart 1986).

### *Cutthroat Trout*

Cutthroat trout have variable life histories and occur as inland and coastal stocks and as anadromous and non-anadromous forms. The stocks and forms vary morphologically, physiologically, and behaviourally according to location and have developed a bewildering array of colours and spotting patterns. Some of the forms have been described as subspecies, and many are still known by common names related to different watersheds or regions (Scott and Crossman 1973). Three subspecies are recognized here, namely the coastal cutthroat trout (*Oncorhynchus clarki clarki*) and two major interior forms, the westslope cutthroat trout (*O. c. lewisi*) and the Yellowstone cutthroat trout (*O. c. bouvieri*). A variety of other subspecies are described elsewhere (Behnke 1992). Common to all cutthroat trout forms are two red, orange, or yellow streaks along the inner edge of the lower jaw on either side, which are the basis for the common name. These streaks are faint in the young and faint to absent in fish in the sea or that have recently returned to fresh water.

*Ecotypes*. Cutthroat trout are distributed in two distinct geographic areas, and this naturally separates them into a coastal and a interior form. The coastal form primarily occurs along the west coast of North America and the interior forms inhabit freshwater systems in the Rocky Mountain and intermountain regions in Canada and the U.S. In some locations the distributions of coastal and interior cutthroat trout overlap (Scott and Crossman 1973). Trotter (1989) recognized three basic life history forms among the coastal populations: "an anadromous form, a potamodromous form that includes both stream-dwelling and lake-dwelling populations, and a non-migratory form that resides in small streams and headwater tributaries." Cutthroat trout of the interior form vary among freshwater systems. Stream-dwelling trout can complete their whole life cycle in the stream of birth or may move to different tributaries in the watershed. Lake-dwelling trout use a lake as nursery and feeding area and return to the home stream to spawn. Cutthroat and rainbow trout readily hybridize and the hybrids are fertile (Scott and Crossman 1973; Nelson and Paetz 1992).

*Geographical Distribution*. Coastal cutthroat trout are distributed over a distance of about 3,000 km along the Pacific coast of North America from Humboldt Bay in California to Prince William Sound in Alaska (Figure 25C) (Behnke 1992). Their distribution ranges inland for about 160 km to the crest of the Cascade Mountains in Oregon and Washington and to the Coastal Mountains in British Columbia and southeastern Alaska (Trotter 1989).

Westslope cutthroat trout are distributed farther inland than the coastal form and occur from the headwaters of the upper Missouri and South Saskatchewan rivers in western Montana and Alberta, to the Arrow lakes of the Columbia River and Shuswap Lake of the Fraser River watersheds, and south to major tributaries of the Columbia River basin in Idaho and Montana. Yellowstone cutthroat are found in the upper Yellowstone River basin and the upper Snake River although they have been introduced elsewhere. Southward, cutthroat trout subspecies' distributions range as far as central Colorado and northwestern New Mexico (Figure 25C) (Scott and Crossman 1973).

*Age and Size.* Cutthroat trout are difficult to age because of variable life cycles and different migration strategies. Their life expectancy is generally between 4 and 7 yr and most migrating adult fish are between 3 and 6 yr old (Johnston and Mercer 1976; Jones 1976; Fuss 1982). Many live only a few years but some have a life span of up to 10 yr. Growth rate in northern populations is slower than in southern ones (Johnston and Mercer 1976). Fuss (1982) reported that age-1 cutthroat trout are about 7.0-7.7 cm and age 2 fish are from 10.0 to 11.3 cm long. Average length and weight for adults is between 30 and 38 cm and 0.5 and 2.7 kg, respectively (Pauley et al. 1989). Sea-run fish range from 0.7 to 1.8 kg. The maximum recorded length and weight for a coastal, non-anadromous stock is 76 cm and 7.7 kg. The angler record for an interior form is a Lahontan cutthroat 99 cm long and 18.6 kg in weight, captured in Pyramid Lake, Nevada in 1925 (Carlander 1969).

*Reproduction.* Male cutthroat trout can spawn for the first time at 2 yr of age whereas females generally do not spawn before age 4 (Johnston 1981; Fuss 1982). In Alaska, most anadromous female cutthroat spawn when they reach age 5 or 6 (Trotter 1989). Lake-dwelling, coastal cutthroat trout generally spawn for the first time at age 3-4. Some fish may spawn every year, whereas others spawn every other year. Many do not spawn more than once because of high post-spawning mortality, but some may return to spawn four or five times (Trotter 1989).

Time of spawning for both coastal and interior cutthroat trout varies with geographic location, but generally takes place in winter and spring from December to May. In southern British Columbia, Washington, and Oregon the peak spawning period is during February. In Alaska, spawning generally occurs in April and May (Trotter 1989). Cutthroat trout from Yellowstone Lake, Montana spawn after ice breakup in spring and early summer in water temperatures near 10°C (Scott and Crossman 1973).

Spawning of inland cutthroat trout takes place in riffles in isolated tributaries of large or small streams with small to moderate size gravel about 0.16 to 5 cm in diameter (Pauley et al. 1989). Water levels over the spawning beds are about 15 to 45 cm deep and the gravel beds generally have a gentle gradient. Lake-dwelling cutthroat trout can spawn both in inlet and outlet streams of lakes (Trotter 1989). Coastal cutthroat trout prefer to spawn in headwater tributaries of larger streams with average flow rates of 0.12 $m^3$/sec and not exceeding 0.28 $m^3$/sec (Johnston 1981). Mean current velocities for spawning westslope cutthroat trout are between 0.30 and 0.37 m/sec (Liknes and Graham 1988). The nests are generally dug in riffles at the tail end of pools in water depths between 10 cm and 1 m (Johnston and Mercer 1976). Average depth of nest sites is 15-45 cm (Trotter 1989).

The spawning ritual of cutthroat trout is typical for salmonids with the female preparing the nest while being courted by the male. The nest depressions dug by the female are 30 cm or more in diameter and 10-13 cm deep. More than one male can take part in the spawning act. After spawning the female moves gravel from upstream of the nest over the eggs to a depth of about 15-20 cm. Spawning takes place during day and night over a period of 2-3 days, during which time several nests are dug in succession until all eggs have been released (Smith 1941). Post-spawning mortalities are sometimes high due to weight loss and rigours of spawning (Scott and Crossman 1973). Sumner (1953) found that in Oregon 39% of the population survived the first spawning, 17% the second, and 12% the third. The fish that survive migrate downstream in early to late spring and the anadromous trout return to the ocean.

There is strong positive linear correlation between length of female cutthroat trout and number of eggs. Fecundity ranges from 226 to 4,420 eggs for females from 20 to 43 cm in total length in Washington (Scott and Crossman 1973) and from 486 to 2,286 eggs per female in Alaska (Jones 1975). Eggs are orange-red and on the average are 4.3-5.1 mm in diameter.

*Incubation and Emergence.* Incubation time is dependent on water temperature and eggs require about 310 temperature units to hatch (Liknes and Graham 1988). According to Scott and Crossman (1973) time to hatching is about 6-7 wk. The alevins stay in the gravel for 1-2 wk after hatching before they emerge as fry. Emergence occurs from April for coastal to August for interior populations.

*Juveniles and Immatures.* Fry are less than 3 cm long upon emergence and quickly move to stream margins with shallow and low-velocity water (Johnston and Mercer 1982). Distribution of the juveniles in freshwater systems is dependent on the presence of other salmonids. If competing species are absent, the juveniles may stay along the stream margins and in side channels throughout the summer (Bustard and Narver 1975). If coho salmon fry occur in the same system then cutthroat fry are usually displaced on to the riffles. Here they will stay until autumn when decreasing temperature reduces aggressive interactions or increased winter flows displaces them downstream (Trotter 1989). Scales appear when the fish reach a fork length of about 3.5 cm sometime during their first summer. After 1-4 yr in small streams, juvenile cutthroat trout move to larger rivers, a lake, or the ocean, depending on the type of population. Many cutthroat trout, however, remain their whole life in small streams, some even in one pool close to the area of birth (Trotter 1989).

Cutthroat trout juveniles that migrate to the ocean go through a smolt transformation, similar to other anadromous salmonids. Age of smolting is variable and can occur between age 1 and 4 or later, but generally takes place between 2 and 4 yr of age (Trotter 1989). The fish have then reached a length of about 13 cm for the earlier ages to 20-25 cm for the older ones (Scott and Crossman 1973; Pauley et al. 1989).

*Movements and Migration.* After their first winter in streams anadromous juvenile cutthroat trout start to range more widely. They may move downstream to the main stem or migrate up- and downstream several times before smolting. Migration to salt water takes place from March to June and peaks in mid-May in British Columbia, Washington, and Oregon (Johnston and Mercer 1976). Before entering seawater, cutthroat trout school, and they remain in groups during their marine life. In the ocean cutthroat trout usually stay within the influence of the river or move only a short distance, generally less than 50 km away from the home stream along the coastline to 31.5 km offshore (Trotter 1989). In sheltered waters, such as Puget Sound and the Strait of Georgia, cutthroat trout tend to stay in shallow coastal waters (<3 m deep) feeding and migrating along the beaches (Jones 1976; Johnston 1981). Some may stay in estuaries and move into streams in spring to feed when the young salmon are migrating; others may remain at sea for one or more years (Scott and Crossman 1973). However, most cutthroat trout do not overwinter in salt water and return to the stream in the same year that they migrated to sea (Pauley et al. 1989).

Not all cutthroat trout migrating back to fresh water from the ocean for the first time spawn that same year. The non-spawners may move into streams other than their natal stream during such migrations. They return to the sea after the winter for a second time before they eventually spawn in their home stream. Anadromous cutthroat trout migrating upstream during autumn and winter in a particular system may thus consist of non-spawners, first-time spawners, and repeat spawners (Trotter 1989).

Non-anadromous juveniles may stay in the tributary of birth, and move to the main stem of the river or a lake after one or more years following emergence. Many of the non-anadromous coastal cutthroat trout populations show similar down- and upstream migratory patterns as the anadromous trout, except that they do not enter salt water. These populations use the main stem of rivers or lakes to feed and grow in a similar way as anadromous fish use ocean resources (Trotter 1989). Lake-dwelling cutthroat trout may stay in tributaries for 1 to 3 or 4 yr before migrating to a lake. They return to the spawning grounds in late winter or spring rather than in autumn and early winter as the anadromous fish. Spawning in these fish takes place from February to early June when water temperatures are 5-6°C (Trotter 1989). The non-migratory cutthroat trout of both coastal and interior forms may exhibit seasonal movements, but such movements generally are restricted and cover only small distances. All cutthroat trout forms generally home with great fidelity to their natal stream.

## THE TROUTS

The two species of trouts considered herein are typical North Atlantic rim fish. Of all the salmonids, the brown trout has the most eastern distribution. This species occurs naturally only on the European side of the Atlantic Ocean, as far east as Russia and Afghanistan. The Atlantic salmon is found in both the eastern and western Atlantic rim countries. Both Atlantic salmon and brown trout can have freshwater and saltwater life history components, although the brown trout is a more freshwater-oriented species whereas the Atlantic salmon is strongly anadromous.

### *Atlantic Salmon*

Atlantic salmon are endemic to the northern Atlantic Ocean and spawn on both European and North American continents. Most of the populations are anadromous and many make long-distance migrations in the Atlantic Ocean between breeding and feeding grounds. Some landlocked populations occur, which use lakes in a similar way as the anadromous fish use the ocean.

*Ecotypes*. The Atlantic salmon breeding in North American and European river systems form many distinct non-interbreeding sub-populations (Thorpe and Mitchell 1981). Each of these sub-populations consists of a number of identifiable ecological forms. Spawning populations are frequently very small, with fewer than 40 pairs of anadromous parents in any spawning class. Saunders and Schom (1985) stated that, "The extremely diverse life history of Atlantic salmon may be the mechanism that allows small populations to persist and maintain genetic diversity".

The Atlantic salmon on the European side can be separated into western and northern components, which apparently occupied different refugia during the last ice age. There is a boreal stock, which was isolated in the North Sea area and a Celtic stock, which remained south of the glaciers. The boreal stock may have been isolated for 15,000 yr and recolonized Scotland, Ireland, and areas farther north after the glaciers retreated. The Celtic stock is considered to have repopulated western Europe and the west coast of Britain, south of the terminal moraines (Thorpe and Mitchell 1981). Within each of the western and northern components there are genetically distinct stock differences. Different stocks with inherited characteristics also occur on the North American side in the eastern United States and eastern Canada (Saunders 1981). As on the European side of the Atlantic, these differences are maintained because of the strong homing tendency of these salmon.

*Geographical Distribution.* Atlantic salmon occur in the northern Atlantic Ocean (Figure 26A). In Europe they spawn from the Duero River in Portugal to the Kola Peninsula in Arctic Russia. On the North American continent they occur in the Ungava region of northern Québec, in eastern Québec around the Gulf of St. Lawrence, throughout Labrador and Newfoundland, in New Brunswick, Prince Edward Island, and southward along the northeastern U.S. coast as far as the Connecticut River. Atlantic salmon also occur in the Baltic Sea where they are often referred to as Baltic salmon. A number of land-locked populations are found in several lakes in southern Sweden, Newfoundland, Labrador, and Québec in Canada, and Maine in the U.S. The land-locked salmon use the lake in a similar way as the anadromous fish use the sea.

Atlantic salmon used to exist in Lake Ontario but have been extinct from this lake since 1900. Many attempts have been made to introduce this salmon in different parts of the world, including back into Lake Ontario, but without much success. A small land-locked population has been established in New Zealand on the south island.

*Age and Size.* Age and size of Atlantic salmon at maturity are strongly influenced by environmental factors. These fish can stay in fresh water for 2-6 yr (generally 2-3 yr) before migrating to sea as smolts and can spend 1-5 yr at sea before returning to reproduce in fresh water. In eastern Canada, Atlantic salmon do not grow older than 9 yr (Scott and Crossman 1973). In the Ungava Bay area of northern Québec, Power (1969) recorded ages of 11 yr. The greatest age reported for Atlantic salmon is 14 yr (Carlander 1969).

Many Atlantic salmon return to spawn after one winter in the sea, varying in size from 1.5 to 4.5 kg. Those that return after two years in the marine environment grow to about twice this size and fish that stay in the ocean for up to 4.5 yr can weigh from 18 to over 30 kg and attain lengths of 120-135 cm. On the average Atlantic salmon grow to a size of up to 18 kg. Those fish that stay entirely in fresh water grow to sizes of 1-2 kg and sometimes much larger.

*Reproduction.* After spending one or more years in salt water, Atlantic salmon return to their home river to spawn and stop feeding when they enter fresh water. In eastern Canada, Atlantic salmon spawn in October and November. In northern Québec, i.e., the Ungava Bay region, spawning can start by the end of September (Power 1969). In Britain spawning can occur as early as September, but usually takes place in November and December in most rivers, and can last well into January. In general, northern populations spawn earlier than southern ones. After a few weeks in fresh water both male and female change colour and shape, the male more pronounced than the female. Both lose their silvery ocean colouration and become rusty-brown on the sides and yellowish brown on the back and head. The head of the male elongates and the lower jaw becomes enlarged and develops a hook or kype (Mills 1986).

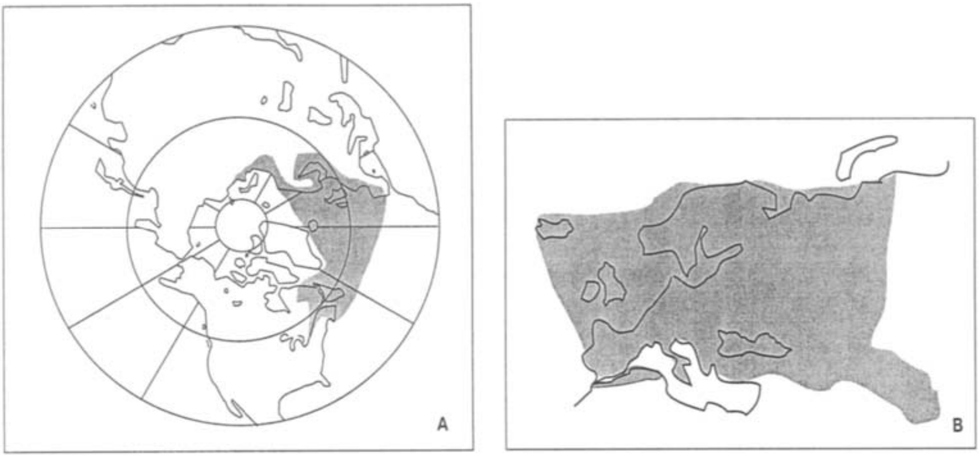

FIGURE 26. Geographical distribution of (a) Atlantic salmon and (b) brown trout.

Atlantic salmon need shallow gravelly areas in streams for spawning and prefer temperatures ranging between 4.4 and 5.6°C (DeCola 1970). The female selects a suitable area of gravel in about 30 cm of water, usually above or below a pool at the downstream end of riffles or at upwellings of ground water (Danie et al. 1984). Here, she digs a nest of about 15 cm deep. The male courts her during the nest preparation process. When the nest is ready, both female and male crouch into the deepest part and release eggs and sperm simultaneously. Soon after the spawning act the female covers the eggs with about 10-25 cm of gravel by gently digging in front of the nest. This operation is repeated several times until all the eggs have been released.

Atlantic salmon that have just finished spawning are called kelts. Some kelts return to sea soon after spawning, while others may overwinter and leave for the sea from March to May. In some cases males overwinter in the river. Many Atlantic salmon die after spawning but some may survive and return for spawning one or more times. Generally, no males survive spawning and only a small proportion of females will go to sea again to return for a second or even third spawning (Mills 1986). The number of second-time spawners varies greatly among rivers, ranging from 0.6 to 34% (Phillips and Rix 1988). Before re-entering the sea the fish will change to their previous silver colouration again.

Fecundity varies greatly among Atlantic salmon females from different river systems. On the average females have from 1,300 to 1,750 eggs/kg body weight. The eggs are pale orange or amber in colour and are about 5-7 mm in diameter. Upon release in the water they are adhesive for a short time (Scott and Crossman 1973).

*Incubation and Emergence.* The eggs incubate in the gravel during the winter. The period of development depends on temperature and ranges from 160 d at 1.1°C, 110 d at 3.9°C, to 90 d at 7.2°C (Menzies 1949). The alevins hatch in March and April and the fry emerge from the gravel in May or June.

*Juveniles and Immatures.* Upon emergence the fry stay in rapid flowing water until they are about 6.5 cm long. The juveniles are called fry until the end of their first year of life starting from the egg, and are then referred to as parr. They may live in streams or shallow parts of the rivers for 1-4 yr, or even longer in the more northern areas, before turning into smolts. The juveniles require stony streams with clear and well-oxygenated water during their few years of life in fresh water. Growth of parr is generally very slow. In the river Shannon in Ireland, the average length of parr ranges from 14 cm for 1-yr olds, 14.7 cm for 2-yr olds, to 16.3 cm for 3- and 4-year-old fish (Went 1955).

In the Maritime Provinces of Canada, parr go to sea as smolts when they are 2 or 3 yr old. In Ungava Bay of northern Québec, parr are 4-8 yr old when they smolt and in Spain, France, and the southern U.K. smoltification occurs at 1, 2, and 3 yr of age (Saunders and Schom 1985). Smoltification is size-dependent and occurs when the fish have reached a certain size. In Britain, smolts start to move downstream when they are about 12.5 cm in length. In eastern Canada smolts are 12.7-15.2 cm long and in the Ungava region in northern Québec they grow to a length of 18 cm (Power 1969). Some parr may become precociously sexually mature during their first, second, or third year. Some may then change into smolts the following year or repeat becoming reproductively active. Female parr have never been found with ripe eggs.

*Movements and Migration.* Movements of parr in fresh water are limited. During the first few months after emergence they may stay close to the nursery area and then move to other parts of the river system. Smolts migrate to sea during April and May. Downstream migration is triggered by a rise in water temperature and increased river flow as a result of rain (Mills 1986). Early in the season, movement occurs primarily during hours of darkness, but by late May and early June, movement also takes place during daylight hours (Mills 1986). Upon reaching the estuary, smolts may hold for a while, probably to physiologically adjust to saline water conditions.

Juvenile Atlantic salmon originating from Canada, the U.K., France, and Spain migrate northward to feeding grounds west, south, and southeast of Greenland. Other feeding grounds are found: (1) off the east coast of Newfoundland where salmon from Canadian rivers have been captured; (2) off the north Norwegian coast where salmon from northern European rivers have been taken; (3) around the Faroe Islands with fish from Britain, Ireland, Iceland and Scandinavia; and (4) off the west coast of Ireland with salmon from British and Irish rivers (Thorpe and Mitchell 1981). Smolts originating from Swedish and Finnish rivers in the Gulf of Bothnia migrate southward to an area around the islands of Bornholm and Gotland in the Baltic Sea. The length of time Atlantic salmon spend feeding in the sea varies. Many come back to spawn after one winter at sea as grilse[4], but most return after two years in salt water. Some salmon can remain up to 4.5 yr in the ocean before returning to spawn. Atlantic salmon that survive spawning and return to sea, either in late autumn or after the winter, do not seem to travel all the way back to the ocean-feeding grounds. They may remain near the river in which they spawned to return to their natal river to spawn within a year.

Atlantic salmon tend to congregate in the estuary before entering the river. They move up and fall back with the tide. Migration upstream only occurs at temperatures above 5°C and if facilitated by flood conditions, which allow the fish to swim across shallows and up rapids. In general, upstream movement is not prevented by low flow conditions, as long as the fish can navigate obstacles in the river (Mills 1986). Soon after entering fresh water Atlantic salmon stop eating and slowly lose weight. Atlantic salmon can enter fresh water all year around. Each run can have fish from different year classes of several brood years, consisting of grilse, 2-seawinter, 3-seawinter, and repeat spawners from each of these three groups (Saunders and Schom 1985).

Atlantic salmon prefer well-oxygenated water and, in slower rivers, lie below rapids or weirs. In a particular river, successive generations tend to occupy the same holding area year after year. Some of these areas are only occupied during high water conditions, whereas others are used most of the time (Phillips and Rix 1988). Atlantic salmon travel day and night during upstream migration. In larger rivers they can cover distances of 40 km/d, but in shorter rivers they tend to remain in pools in the lower reaches or below rapids. Many salmon rivers have lakes, which provide good resting opportunities especially in times of low water. They may remain in about 3 m of water along the lake shores or in shallows.

The leaping behaviour of Atlantic salmon during migration upstream is spectacular. The word salmon is derived from the Latin word for leap. Waterfalls to 4 m high can be cleared if water conditions are right. Leaping may also occur when salmon resting in pools are disturbed, when they are preparing to leave pools, and spontaneously during migration.

In general, fish that return before November will spawn in the same year, whereas those that arrive later will remain almost a whole year in the rivers before they spawn (Phillips and Rix 1988). In spring, grilse and older salmon migrating upstream and kelts moving downstream can be travelling in opposite directions in the same river system. Fish that have just returned from the sea may join aggregations of kelts in the same resting pools for a while, before each go their own way.

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[4] Grilse are salmon that have spent a little over a year at sea. They appear to originate from parr that have spend 3 or more years in the river.

### *Brown Trout*

Brown trout are a variable species endemic to Eurasia. They spawn in small streams and may occupy the sea, a lake, or stay in the river during their life history. The variety of life history forms and shapes, sizes, and colouration has formerly led to recognition of several different species and subspecies. All are now considered ecotypes of the same species, which as a result of ecological adaptations developed into many distinct forms. The anadromous form is often called sea trout.

*Ecotypes*. Brown trout occur in three major ecological forms: riverine, lacustrine, and anadromous forms. Within each of these forms, they vary with respect to shape, size, colour, and detailed life history traits. The greatest variation occurs among the lake-dwelling populations. Several forms of brown trout with irregular dark blotches are recorded from isolated populations in North Africa, the islands of Corsica and Sardinia, southern Greece and the Taurus mountains of Turkey. Ten other distinguishable forms are found in the eastern Alps, in Yugoslavia, and in northern Greece. This richness in ecological forms has led to some confusion in the taxonomic literature and many of the different types have been classified as subspecies and, in some cases, even as separate species. Lately, it has been recognized that the variety of forms are the result of ecological adaptations and that the morphological differences are the result of different feeding, water chemistry and colour, colour of bottom, and other environmental conditions (Elliot et al. 1992; Phillips and Rix 1988).

Natural hybrids frequently occur between brown trout and Atlantic salmon but are often difficult to identify, except by modern biochemical methods. Brown trout also hybridize with brook charr, which results in a strikingly beautiful fish called the tiger trout.

*Geographical Distribution*. The natural distribution of brown trout includes Iceland, the British Isles, and continental Europe ranging from Spain eastward to Afghanistan and Russia and northward to Norway and northern Russia (Figure 26B). In the south brown trout occur in the countries bordering the Mediterranean Sea, both on the European and north African sides (MacCrimmon and Marshall 1968). Large brown trout occur in most of the large lakes in the Alps and Carpathian Mountains, and in many lakes in Scandinavia and Iceland. They live with and prey on charrs and whitefish.

Brown trout have been transplanted to many parts of the world; e.g., North and South America, Africa, Asia (India, Pakistan, and Japan), and Australia and New Zealand. Introductions to the U.S. (Maine) started in 1883, and were followed a year later with egg shipments to Canada (Newfoundland). The introductions were primarily made into stream and river habitats but over time many of the introduced stocks have developed lake-dwelling and sea-run forms.

*Age and Size*. Growth of brown trout is dependent on food supply. In acidic lakes where food is scarce, sizes of brown trout may range from 0.2 to 2 kg. In alkaline and shallow lakes in which food conditions are favourable, sizes of 2-3 kg may be reached in about 7 yr. Sizes of up to 4.5-6.8 kg have been recorded in Britain under natural conditions. The largest brown trout known was captured in Loch Awe, Scotland in 1866, and was 102.9 cm long and weighed 17.8 kg (Scott and Crossman 1973). Lacustrine brown trout in Britain usually are 10-13 yr old when captured and weigh between 3 and 12 kg. Some anadromous forms can be as old as 23 yr (Phillips and Rix 1988). In southern Ontario brown trout attain a total length of 16.5 cm at age 1, 24.9 cm at age 2, 38.8 cm at age 3, and 42.7 cm at age 4. Sea-run brown trout are 30-40 cm long (0.4 kg) after 1 yr in the sea, 40-50 cm (1-1.5 kg) after 2 yr, and 50-60 cm (1.5-3.5 kg) after 3 yr (Mansell 1966).

The age of sexual maturity for brown trout is not well established. It differs in populations among lakes and varies inter-annually depending on food conditions (Pyefinch 1960). The range at which males and females mature probably varies from 2+ to 5+ and from 3+ to 6+ yr of age, respectively. Large anadromous brown trout in Britain are considered to be 7-10 yr old.

*Reproduction*. Spawning of brown trout takes place in late autumn to early winter (Scott and Crossman 1973). Temperatures at which spawning begins range from 6 to 9°C, but may be as high as 12.8°C (Raleigh et al. 1986). Brown trout generally choose spawning sites in small streams at the head of riffle areas or on the downstream end of pools, where the gravel slopes upward. These sites are characterized by water freely flowing through the gravel and by upwelling water (Benson 1953). Gravel size of spawning substrate may range from 0.3 to 10 cm with a preference for sizes from 1 to 7 cm. For optimal spawning conditions the gravel substrate should have less than 5% fines; 30% or more fines results in low survival rates of embryos and emerging fry (Raleigh et al. 1986). Water depth at nest sites ranges from 15 to 90 cm. Optimal depths are from 24 to 46 cm with the preferred depth around 31-32 cm (Raleigh et al. 1986). More important than water depth is water velocity over the nest site. Suitable velocities are from 15 to 90 cm/sec and optimum levels range between 40 and 70 cm/sec (Raleigh et al. 1986).

Spawning behaviour of brown trout is similar to that of most salmonids. The female chooses the nest site and prepares the nest. The male courts her while she is digging the nest and he defends the nesting territory against intruding males. When the nest is finished, female and male simultaneously deposit ova and sperm during a short spawning act. The female then covers the eggs with a layer of gravel. This activity continues into the excavation of the next nest. Brown trout nests vary from 30 to 107 cm in width and from 7.6 to 30.5 cm in depth (Raleigh et al. 1986). Several nests are generally completed in succession by the female during the spawning season before she is spent, with each nest containing a few hundred eggs. She then finishes off the redd mount containing the egg pockets by digging over the whole area and defends the redd against other females searching for a place to spawn.

When the female is spent she will move downstream. Males generally stay on the spawning grounds a little longer looking for other opportunities to spawn. In the case of lacustrine populations, spawned-out males and females may move back into the lake. The spawned fish from anadromous populations that survive stay in the river over winter and move downstream to salt water during the following spring (Pyefinch 1960). If stream conditions prohibit lake-dwelling brown trout from entering the spawning stream they will spawn along the shores of the lake. However, mortality among eggs deposited in shoreline spawning areas is generally very high (Pyefinch 1960).

Fecundity of brown trout depends on the size of the female; generally larger females have more eggs. Scott and Crossman (1973) noted that in the Cowichan River of British Columbia, the average number of eggs for 5 and 6 yr old females was 2,020. Maximum fecundity can be as high as 10,000 eggs per female. Eggs are generally amber in colour and have a diameter of about 5-6 mm (Scott and Crossman 1973).

*Incubation and Emergence.* Duration of incubation and time of emergence are dependent on temperature. Raleigh et al. (1986) reported that the optimum temperatures for embryonic development are from 2 to 13°C and that the tolerance level ranges from 0 to 15°C. Average incubation time from fertilization to hatching of the eggs in the gravel is about 2 months, with a range of 134 d at a temperature of 1.9°C to 34 d at 11.2°C . The alevins stay in the gravel until the yolk sac has almost been absorbed. The fry usually emerge in March.

*Juveniles and Immatures.* For the first few days after emergence fry are unable to swim in mid-water so capture their prey by dashing upwards from the bottom toward food organisms that float by with the current. Only later, after the swim bladder has developed and has been filled with air, are the fry able to swim in mid-water. Soon after emergence the fry become aggressive and set up feeding territories. For 3-4-cm fry the minimum distance at which they tolerate other brown trout fry is at least 7.5 cm (Pyefinch 1960). When active feeding has been established the fry disperse to suitable habitat in the natal stream in pools, rocky substrates, and edges of riffles. Sometimes they also occupy the margins of a river, but rarely do they occupy still muddy backwaters or areas with small gravel substrate. The preferred stream areas have water velocities of less than 15 cm/sec. The optimal temperature for free-feeding brown trout fry is assumed to be between 7 and 15°C with a tolerance range between 5 and 25.5°C (Raleigh et al. 1986). Mortality among fry is high during the first few months of stream life. Survival from fry to yearling has been estimated by Mills (1971) at 2.7%.

Juvenile brown trout in streams and rivers take up position in pools, deep riffles, and undercut banks, and move into shallower water during the dusk period to feed. In lakes they keep to shallower water and come to the surface during evening twilight or when aquatic insects are hatching. Lake habitats for brown trout are characterized by clear, cool to cold deep lakes that are typically oligotrophic and that have tributary streams with gravel substrates for spawning. In winter, when temperatures drop to between 4 and 8°C, brown trout tend to move into deep, low-velocity pools to hide (Hartman 1963). The adaptive significance of this winter cover response is to avoid predation, downstream displacement, physical damage from ice, and to conserve energy (Raleigh et al. 1986).

Trout and charr species occupying the same stream use different habitats from headwater areas downstream. Brook charr and cutthroat trout tend to occupy the colder, swifter, less fertile headwater regions, rainbow trout the mid-region of the river system with intermediate habitat conditions, and brown trout the deeper, lower velocity, warmer, more fertile downstream regions (Raleigh et al. 1986).

*Movements and Migration.* Anadromous brown trout or sea trout remain in streams and rivers as parr for 2-5 yr and then migrate to sea as smolts in spring. In Britain most have left fresh water by early June. They generally do not overwinter in the sea and many return to fresh water after 3 or 4 months in salt water. These young trout do not spawn but winter in the river and then return to sea in spring. After this they tend to re-enter the river yearly (Pyefinch 1960).

Juveniles from riverine populations generally stay in the natal stream for a few years before moving away in spring. Movement away from the nursery stream appears to be size related. The bigger the fish the earlier they migrate away. The movements of lacustrine brown trout from nursery stream to lake compare with the seaward migration of anadromous brown trout smolts (Pyefinch 1960).

Brown trout may spend from 1 to 4 yr at sea, with northern populations staying in salt water longer than southern ones (Whitehead et al. 1984). At sea they generally stay in the area of the home river but have been reported to make journeys as far as 300 to 600 km (Whitehead et al. 1984). In the Baltic Sea they have been found 200 km away from the home river. Anadromous brown trout enter the rivers to spawn during the summer. The larger ones enter first in May or even earlier, and the smaller ones later mainly in July or August. Many continue to feed when they enter the rivers but at a reduced rate, and stop feeding prior to spawning. The reduced feeding rate may be due to poorer feeding conditions in fresh water as compared with the ocean (Phillips and Rix 1988).

## THE CHARRS

The distribution of the charrs is Holarctic and includes the most northerly distribution of any fish found in fresh water (Behnke 1980). Charrs have undergone rapid morphological and ecological divergence during recent geological times and that has resulted in a bewildering variety of forms and types. Zoogeographic evidence suggests that an early Pleistocene branch of the genus became established in North America and eventually gave rise to lake charr and brook charr. The lake charr developed into a large lacustrine predator, whereas the brook charr evolved in a more generalized way, inhabiting a wider range of environments (Behnke 1984). Evolutionary divergence of the other *Salvelinus* forms resulted in the development of the Dolly Varden charr and the Arctic charr species groups. This divergence may have taken place during an early Pleistocene glacial period that divided the common ancestral charr into a southern (precursor of the Dolly Varden) and a northern (precursor of the Arctic charr) species group in the Pacific and Arctic ocean drainages (Behnke 1984).

The charrs primarily inhabit fresh water but they also have anadromous forms. The anadromous stocks typically occur in more northern regions than the freshwater ones. In these northern regions, however, resident non-migratory charr can occur in the same river systems with anadromous stocks (Behnke 1980).

### *Brook Charr*

The brook charr or brook trout is indigenous to eastern North America and is the most generalized and adaptable of the charrs. Dispersal and separation into geographically isolated breeding stocks in different habitats after the last ice age has contributed to making this species very variable. This variability is reflected in differences in colouration, growth rate, life history strategies, habitat characteristics, and other features.

*Ecotypes*. Brook charr occur in two basic ecological forms: a short-lived (3-4 yr), small (20-25 cm) form typical of small cold streams and lake habitats, and a long-lived (8-10 yr), large (4-6 kg), predaceous form living in large lakes, rivers and estuaries (Raleigh 1982). The smaller, short-lived form is typically found south of the Great Lakes region and south of northern England. The larger form is distributed in the northern portion of its native range (Behnke 1980). The life histories of these two forms vary because they are adapted to different environmental conditions.

*Geographical Distribution*. The brook charr occurs naturally only in northeastern North America (Figure 27A). Its geographical distribution in eastern Canada ranges from the Maritime Provinces, westward as far as northeastern Manitoba, and northward to the Arctic circle. In the U.S. its range includes the New England states and southward through Pennsylvania, along the crest of the Appalachian mountains to northeastern Georgia, and west to the upper Mississippi and Great Lakes drainages and Minnesota (MacCrimmon and Campbell 1969; MacCrimmon et al. 1971; Scott and Crossman 1973). Brook charr have been introduced widely in many freshwater systems in western North America and other parts of the world, such as South America, New Zealand, Asia, and

Europe (MacCrimmon and Campbell 1969). Many of these introductions have resulted in self-sustaining populations.

Brook charr inhabit small headwater streams, large rivers, ponds, and large lakes in inland and coastal areas. They are found in clear, cold, spring-fed water that is silt-free and has rocky substrate in riffle areas. In areas south of Canada, they tend to occupy headwater stream areas, especially when rainbow and brown trout occupy the same river system. In the northern portion of their native range they occur more in the large rivers (Behnke 1980).

*Age and Size.* Brook charr usually mature at age 3, some at age 2, and generally live 4-5 yr and seldom more than 8 yr. Maximum ages recorded occur in slow growing northern stocks and can range up to 12 yr of age for an anadromous stock from the Koksoak River (Power 1980). Weight and length ranges are: 8.8-15.5 cm and 8.5-42.5 g for age 1+ fish; 12.4-29.5 cm and 22.7-213 g for age 2+ fish; 17.2-38.6 cm and 59.5-581 g for age 3+ fish; 21.2-43.9 cm and 111-848 g for age 4+ fish; 25.1-48.6 cm and 187-1,236 g for age 5+ fish; and 29.4-53.1 cm and 309-1,616 g for age 6+ fish. Fish over 4.5 kg are rare; the largest brook charr caught was 6.6 kg (Scott and Crossman 1973).

*Reproduction.* Brook charr typically spawn in streams or in gravel surrounding spring-upwelling areas of lakes and ponds. Spawning time varies with latitude and temperature. It occurs from late summer (late August or September) in the northern part of their range to early December in the southern part (Raleigh 1982). Over most of the range, October is the usual time of spawning (Power 1980). Males usually arrive first on the spawning grounds. They develop a small kype on the lower jaw. Some territorial behaviour may be evident but aggressive displays increase when the females arrive. The females are less bright than the males and have swollen abdomens full of ripening eggs. Brook charr females remain in deeper water for eggs to ripen before moving on to the spawning grounds to select a nest site. The selection of spawning areas in streams and ponds is more determined by upwelling groundwater than by substrate size (Power 1980; Raleigh 1982).

Spawning of brook charr takes place primarily during daytime. Spawning behaviour is similar to that of *Oncorhynchus* and *Salmo* species in general outline but differs in detail (Smith 1941). For example, during spawning there are usually several extrusions of eggs and milt followed by resting periods before the nest is closed. Covering the nest is done by sinuous movements that sweep the eggs into spaces between the gravel and cover them with small gravel. These egg sweeping and covering movements, which may last for 5-20 min, gradually change into normal digging motions that pile up gravel onto the nest site. This nest cover digging may continue for well over an hour (Power 1980). Females become very aggressive during the post-spawning period and defend the redd area against possible intruders. Brook charr can successfully spawn in gravel beds in lakes as long as oxygen levels are high and the gravel and nest areas are free of silt. Suitable spawning gravel ranges from 3 to 8 cm with less than 5% fines

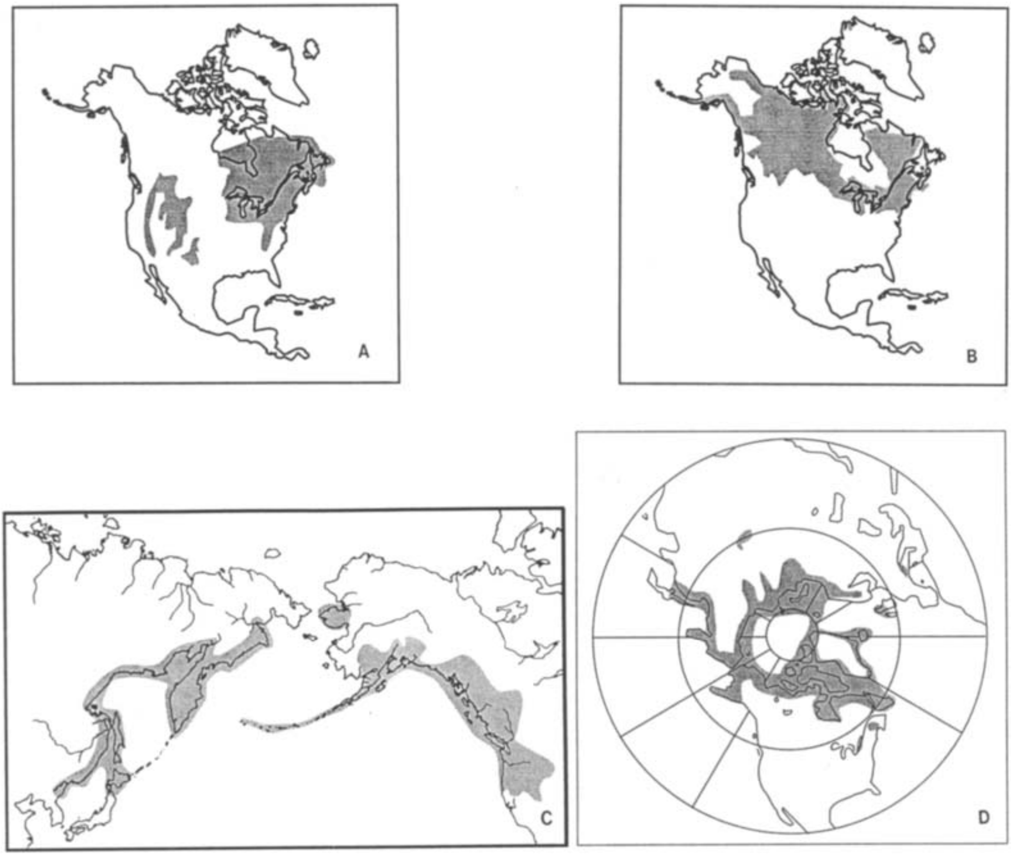

FIGURE 27. Geographic distribution of (A) brook charr, (B) lake charr, (C) Dolly Varden charr, and (D) Arctic charr.

(Raleigh 1982). The preferred temperature range for spawning is from 4.5 to 10°C and spawning does not occur above 16°C (Power 1980; Raleigh 1982).

The number of eggs per female depends on size and varies from 100 for a female 14.4 cm long to 5,000 for one 56.5 cm in fork length. The diameter of the eggs ranges from 3.5-5.0 mm (Scott and Crossman 1973). Under natural circumstances the majority of ripe eggs are shed and egg retention is low. Also, the percentage of fertilization and the viability of eggs under natural conditions is high (Power 1980).

*Incubation and Emergence.* Survival of embryos and alevins depends on water velocity and the intergravel environment. Optimum temperatures for egg incubation range from 4.5 to 11.5°C. Time of incubation ranges from 219 to 490 degree-days for temperatures from 1.5 to 6°C. The upper lethal temperature for developing eggs is about 11.7°C (Power 1980). Brook charr fry emerge from gravel beds from January to April, depending on the local temperature regime and become free-swimming when they are about 38 mm long (Raleigh 1982).

*Juveniles and Immatures*. After emergence brook charr fry disperse into shallow water and move upstream or downstream until territories are established. As they grow older they defend larger territories in deeper waters. Fry of lake-spawning brook charr concentrate in the shallow waters around the lake. Brook charr prefer streams with well vegetated banks and with relatively stable water flows, temperature regimes, and stream banks (Raleigh 1982). In lakes they primarily occupy inshore areas and prefer waters less than 7.5 m deep (Power 1980).

The optimum temperature range for brook charr growth and survival is between 11 and 16°C. They do poorly in streams where water temperatures exceed 20°C for extended periods of time. When temperatures rise in the stream they tend to move to larger bodies of water downstream or into a lake. When already in lakes they may move to greater depth when waters warm up (Raleigh 1982). Brook charr prefer streams with flows of 7-11 cm/sec and, in general, do not occur in streams with water velocities greater than 15 cm/sec (Raleigh 1982). They normally require high levels of oxygen concentrations (≥7-9 mg/l) and do best in waters with oxygen levels near saturation (Mills 1971; Raleigh 1982). Production of brook charr is generally higher in waters with high alkalinity and high specific conductance, although they do occur in waters with a wide range of these two factors (Raleigh 1982). Brook charr appear to be more tolerant of acidic conditions than other salmonids (Swarts et al. 1978).

*Migrations*. Brook charr only make a few habitat changes during their life history. The southern freshwater stocks generally do not migrate far from the spawning grounds. Some may spend their entire lives within the area of the spawning stream or may move up- or downstream for short distances because of intra-specific aggressive interactions (Hunt 1965). They may also make short seasonal migrations to avoid temperature extremes (Scott and Crossman 1973). Movements can occur during every month of the year but are greatly reduced during mid-summer and mid-winter. A part of the population in coastal streams of eastern Canada and northeastern U.S. may go to sea for short times (1 month) during the summer months when they are age 2 or 3, or rarely at age 1 (White 1940; Smith and Saunders 1958). Brook charr return again to fresh water for overwintering. Anadromous stocks of more northern geographic areas migrate seaward in autumn and return to fresh water from April to early June (Power 1980).

### *Lake Charr*

Lake charr or lake trout are indigenous to northern North America from the Atlantic to the Pacific Ocean coasts and typically live in large, deep, generally oligotrophic lakes. They are the largest and become the oldest of the salmonids. In the Arctic they exist in shallow bodies of water and in the Canadian Shield they are abundant in lake depressions scoured out during the Pleistocene glaciation. Spawning of lake charr typically occurs on shoals and along lake shores but, occasionally, they will breed in tributary or outlet streams of lakes. No anadromous forms exist, although some lake charr have been captured in coastal areas in low salinity waters (Martin and Olver 1980).

*Ecotypes*. Lake charr are an extremely variable species with many different sub-populations, which differ in life history strategies, behaviour, feeding habits, colouration, body size, and weight. A number of distinct sub-populations exist in Lake Superior; one of them is called the siscowet and is found in deep waters of the lake. It was once considered a subspecies, *Salvelinus namaycush siscowet*. Because a number of intermediate forms occur between typical lake charr and siscowet, this systematic classification has not been widely accepted (Scott and Crossman 1973).

A fertile hybrid, called a splake or wendigo, is produced by fertilizing lake charr eggs with brook charr sperm, and has been planted in many freshwater systems of North America, especially in Ontario (Scott and Crossman 1973). Splake are intermediate in colouration between the two parents, grow rapidly, and mature earlier than lake charr.

*Geographical Distribution*. In Canada, the natural geographic distribution of lake charr covers the entire country including several Arctic islands (Southhampton, King William, Victoria, and Banks islands) (Figure 27B) (Carlander 1969; Martin and Olver 1980). They are absent from the Hudson and James Bay lowlands, insular Newfoundland, Prince Edward Island, and Siberia. Salt water may have prevented lake charr from spreading to the latter three areas (Martin and Olver 1980). In the U.S., native populations of lake charr occur in New York, Pennsylvania, New Hampshire, Vermont, Maine, Michigan, Wisconsin, Minnesota, Montana, and Alaska (Figure 25B) (Carlander 1969; Martin and Olver 1980).

Lake charr have disappeared completely from Lake Michigan and Lake Ontario, and have become extremely limited in Lake Huron. The disappearance from Lake Ontario appears related to the unavailability of deepwater sculpins (*Myoxocephalus thompsoni*), which were an important food source of lake charr in this lake, in combination with sea lamprey (*Petromyzon marinus*) predation and overexploitation. The sculpins vanished about 1959-1960 for unexplained reasons (Scott and Crossman 1973). Large scale stocking programs have been undertaken by Canadian and U.S. agencies in an attempt to re-establish lake charr populations in the lower Great Lakes (Eshenroder et al. 1984). Many introductions of lake charr have been made throughout North America and into lake systems in Europe, New Zealand, and South America.

*Age and Size*. Lake charr are long-lived fish and reach weights greater than any of the other salmonids. The oldest fish recorded in the U.S. was 28 yr old. In Canada the maximum age ranges from 36 to 40 yr old. Splake reach sexual maturity at about age 3 and have a life span of at least 9 yr (Martin and Olver 1980).

Fork lengths of lake charr range from 14.2 cm at age 0+ (weighing 23 g) to 96.8 cm at age 23+ (weighing 11.1 kg) in Great Slave Lake, Northwest Territories, Canada. In Great Bear Lake, fork lengths range from 2.8 cm at age 0+ to 69.8 cm for age 23+ and fish older than 23 yr have fork lengths from 71.9 to 96.5 cm (Scott and Crossman 1973). Lake charr from many Canadian lakes can reach weights in excess of 22.7 kg (50 lbs) and in some cases exceed 45.4 kg (100 lbs). In Lac la Ronge, Saskatchewan, the oldest captured lake charrs were 17-18 yr old and averaged 88.9 and 91.9 cm in fork length, respectively. The 46.3-kg record lake charr from Lake Athabaska was 126 cm long and between 20 and 25 yr old (Scott and Crossman 1973).

The growth rate of lake charr is extremely variable and varies in different lake systems depending on season, lake area, stock, age, and types and densities of food. Growth rate tends to be slower in the northern part of the distribution range. Also, lake charr feeding on fish grow more quickly than those feeding on plankton (Martin 1966).

*Reproduction.* Age at first sexual maturity of lake charr is related to growth. It varies from 4 to 19 yr and is usually attained at about age 6 or 7 (Scott and Crossman 1973). In rapid growing populations the males can mature at age 4-5 and the females at age 6-8 (Carlander 1969). Males usually mature one year earlier than females. Feeding rate decreases as the breeding season approaches and the lake charr move into rocky shallows or shoal areas in preparation for spawning. When once common, Lake Simcoe lake charr would arrive on the breeding grounds between 9-19 d before spawning (MacCrimmon 1958).

Lake charr spawn primarily in lakes, less often in tributary streams and rivers as in isolated locations in Lake Superior, and in rivers of the Canadian tundra and Alaska (Loftus 1957; McPhail and Lindsey 1970). In general, the period of spawning of northern populations occurs from mid-August to mid-September and for southern populations from September to December. In large lakes, such as Lake Superior, different stocks may spawn at varying times. Spawning appears to be triggered by declining temperatures and photoperiod coupled with strong on-shore winds (Carlander 1969; Martin and Olver 1980). Critical temperatures for spawning range from 4.5 to 14°C for different lakes (Marcus et al. 1984) and in many of the more temperate lakes, spawning starts when temperatures drop to about 10°C (Martin and Olver 1980). In some New York lakes, cloudy days during July, August and September in combination with lower temperatures significantly advances the time of spawning (MacCrimmon 1958).

Both temperature and wind are important in determining the length of spawning. Heavy on-shore winds lasting for several days can induce and shorten the time of spawning, whereas long periods of calm and bright weather may extend both prespawning and spawning periods. Lake charr have been observed to stay on the spawning grounds from 18 d to 4 wk. However, actual egg deposition and fertilization may take place within 2 d under the right wind and other weather conditions. Spawning may occur each year after fish reach maturity, as in Great Slave Lake, or intermittently every second or third year, as in Great Bear Lake. Martin and Olver (1980) indicated that the reason for this interrupted reproductive pattern is not well known, but suggested that it could be related to different patterns of light and darkness in high latitudes.

Although lake charr can spawn over a variety of substrates, they prefer broken rubble or angular rock 3-15 cm in diameter (Martin and Olver 1980). Other types of spawning substrates can range from clay bottoms in southern Lake Michigan, to silt, hardpan, clay, marl, and gravel in Green Lake, Wisconsin, to stone bottoms in Lake Superior (Carlander 1969). In general, the beds are free of mud, sand and detritus. Spawning takes place in depths ranging from 0.15 to 6 m in smaller inland lakes to over 60 m in Seneca Lake, New York, and 90 m for siscowets in Lake Superior (Martin and Olver 1980). In the Great Lakes, spawning generally occurs at depths less than 36 m.

Spawning of lake charr occurs during evening hours, with the fish appearing on the grounds soon after dusk and departing again before midnight. Males generally arrive first followed by the females. Splake spawn during both night and day, which combines the day and night-time spawning behaviour of brook and lake charr, respectively (Scott and Crossman 1973). Lake charr do not dig a nest but clean rubble and boulders by brushing them with the body or tail fin, or by rubbing them with their snout. This behaviour cleans debris, silt, algae, and slime from the stone surfaces. Martin (1957) and DeRoche (1969) did not observe any intentional cleaning of the spawning shoals; presumably many areas are cleaned by wave action and currents.

The spawning behaviour of lake charr appears different from that of most salmonids, probably because this is the only species that primarily spawns in lakes. Martin and Olver (1980) described the courting and spawning of lake charr as follows: "After arrival of the females on the beds, the males begin courting and nudging their sides, swimming beneath them, brushing their vents with the dorsal fins, and by nipping in the region of the anal fin and gills. A male does not restrict these attentions to a particular female, but courts any that comes within range. At about this time, the shiny dark areas appear on the sides and around the heads of the males. The actual spawning act ... consists of one or more males pressing against one or several females for a few seconds, with mouths agape and the dorsal fin of the male erect. Although milkiness has been observed in the water signifying the emission of sperm no eggs have been seen in the process of being shed. The brief nature of the act suggests repeated oviposition by the female."

Eggs can be deposited anywhere in the cleaned area. The fertilized eggs are not covered or buried but fall into crevices between the rocks, a process that is assisted by the sweeping motions of the female with body and fins. There is also no guarding of the nest site as in other salmonids (Martin and Olver 1980). Fertilization rate is considered high (73-92%) in lake charr (Martin and Olver 1980). After spawning, lake charr may stay in the area or disperse throughout the lake and travel several hundred kilometres away.

Three sizes of ova occur in mature female lake charr, representing the eggs for three yearly spawnings. The largest are spawned during the most immediate spawning, and the intermediate and smallest sizes during the following two years of spawning. The sizes of ripe eggs in lake charr range from 3.7 to 6.8 mm in diameter, with an average of about 5 mm. The average number of eggs per female unit weight for different lake systems ranges from 628 to 3,135/kg. Total number of eggs per female increases with size of fish from about 400 for small fish (38.1 cm), to 21,500 for large fish (109 cm and 16 yr old) (Martin and Olver 1980).

*Incubation and Emergence*. The eggs of lake charr incubate through the winter in rock and boulder crevices. The length of incubation ranges from 2 to almost 6 months for different lake systems, but usually lasts 4 to 5 months (Martin and Olver 1980). Hatching generally takes place in March or April, but can occur as late as June as in Great Bear Lake. Length of the alevins at time of hatching ranges from 23 to 33 mm. They may stay in the rocky crevices after hatching for another 1-2 months until the yolk sac has been absorbed. The fry emerge from May to June. Before leaving for deeper waters the fry make a trip to the surface to fill their swim bladder. Fry from stream-spawning populations leave the rivers for lakes soon after hatching, possibly to avoid high light intensities when there is a lack of ice cover (Paterson 1968).

*Juveniles and Immatures*. The biology of young lake charr is not well known. Young-of-the-year and older lake charr tend to stay in the deeper waters of lakes (Marcus et al. 1984). In far northern lakes, such as Great Bear Lake, the fry may remain in inshore waters along rocky shorelines for months or even years. Growth of the young varies greatly in different lake systems. In the middle portion of the Canadian range growth is rapid. In spring, after ice break-up, lake charr often occur in surface waters. As spring advances and water temperatures rise, they move to cooler waters between 5 and 7°C (Martin and Olver 1980). They return to shallower waters during the autumn as temperatures decrease and stratification breaks down (Rawson 1961).

Martin and Olver (1980) reported that lake charr lakes are generally large, higher in altitude, deeper, clearer, colder, better oxygenated, more acidic, softer, and lower in alkalinity, buffer capacity and total dissolved solids than lakes without this species. Lake charr are the least tolerant of salt water but have been captured in coastal waters with a salinity between 6 and 9‰. Their upper limit of salinity tolerance is between 11 and 13‰. The depth distribution of lake charr in inland lakes is primarily related to water temperature (Martin and Olver 1980). They usually stay below the thermocline during the summer months, which in many small lakes is at depths of 12-18 m (Scott and Crossman 1973). In large lakes, such as Lake Superior, lake charr are found at depths ranging from 18 to 53 m (Dryer 1966) and they have been captured as deep as 215 m and

400 m in Reindeer lake, Saskatchewan, and Lake Tahoe, California, respectively. Lake charr do not form schools, but they sometimes occur in aggregations in response to certain environmental conditions. These aggregations are most common during the early years of life. Large lake charr are relatively solitary (Martin and Olver 1980).

*Movements and Migrations*. Movements of lake charr differ among stocks. Some stocks are nomadic whereas others are more sedentary. In large lakes, lake charr can travel great distances. Fish tagged in Lake Superior travelled between 117 and 306 km within a month (Eschmeyer et al. 1953). However, most lake charr in this lake stay within 48 km of their spawning areas. In Great Bear Lake, movements ranged from 32 km in one year to no obvious movement for 5 or 6 yr (Johnson 1975). In general, smaller fish tend to be less migratory than larger ones (Eschmeyer et al. 1953). Temperature is probably the single most important factor determining movements of lake charr, but the search for food may also play an important role. Homing to the same spawning beds year after year appears to be the general pattern in lake charr, although, in some lake systems homing behaviour is not evident (Martin and Olver 1980).

### *Dolly Varden Charr*

The Dolly Varden charr, or simply Dolly Varden, closely resembles Arctic charr and at times has been classified as a subspecies of the Arctic charr. Present consensus is that these two charrs are separate species. Wherever Dolly Varden and Arctic charr occur sympatrically over broad areas of Asia and North America, they remain reproductively isolated (Scott and Crossman 1973; Armstrong and Morrow 1980). The two species exhibit sharp differences in life history and taxonomic characters with no hybridization. Dolly Varden of Alaska are anadromous, overwinter in lakes, and spawn in tributaries (or are resident in tributaries) to the lakes, whereas Arctic charr are strictly lacustrine and more piscivorous. Phenotypically, Dolly Varden have smaller spots on the body, a blunter nose, fewer gill rakers, and fewer pyloric caeca than Arctic charr (DeLacy and Morton 1943; McPhail 1961; Behnke 1984).

*Ecotypes*. Dolly Varden charr have adapted to various freshwater and saltwater habitats within their geographic distribution range and occur in a variety of ecotypes (Armstrong and Morrow 1980). Two ecological forms can be distinguished, a northern and a southern form. The separation probably occurred during the glacial period, following the separation of Dolly Varden and Arctic charr (Behnke 1984). Northern and southern forms do not appear to occur sympatrically.

Both northern and southern forms of Dolly Varden charr have freshwater and anadromous types. The southern form, which occurs in California, Nevada, Montana, and Idaho, tends to stay more in fresh water during its life cycle. In the northern form, which occurs from Washington north, anadromy is more pronounced (Behnke 1984).

Most anadromous types only stay in salt water for a short time during summer and generally spend the winter in fresh water (Armstrong and Morrow 1980).

The strictly freshwater types of the southern form can be separated into those residing in lakes and those inhabiting stream-lake systems. In addition, some populations of non-anadromous Dolly Varden charr are found in large rivers and lakes of the Pacific Northwest including southern British Columbia and Alberta. The southern interior forms generally are now called bull charr or bull trout as taxonomists consider them a separate species, *Salvelinus confluentus* (Cavender 1978; Behnke 1980).

The freshwater types of the northern form can be separated in stream-resident, lake-resident, and spring-resident stocks. Stream-resident stocks in Alaska occur in headwaters or small, clear-water tributaries of major rivers. They grow slowly, generally stay under 30 cm long and retain parr marks and other juvenile characteristics during their life cycle. They mature sexually between ages 2+ and 4+ in small river systems, and between 4+ and 6+ in large systems (Armstrong and Morrow 1980). Lake-resident stocks occur in some lakes in Kamchatka (Lakes Ushovskoye and Azabachye) and in Alaska in the upper Canning River drainage area and complete their life cycle in lakes. They grow faster than other freshwater populations and can reach lengths ranging from 40 to 75 cm. Also, they do not spawn annually and have a lower fecundity than anadromous fish (Armstrong and Morrow 1980). The spring-resident stocks are known from isolated springs in northern Alaska, and the Yukon and Northwest Territories in Canada. The fish grow slowly, are small in size, mature early, spawn later (mid-October, November), and have smaller eggs (average >3-3.8 mm) than Dolly Varden charr in other habitats (Armstrong and Morrow 1980).

Armstrong and Morrow (1980) summarized the ecological characteristics of the three freshwater types of the southern form as follows: "Each of these groups has different habits and its life history is adapted to its particular environment. The stream resident form seems to have adapted to its environment by its small size, early maturity, reduced fecundity, and possibly, shortened life span ... Stream-lake resident Dolly Varden charr appear initially to live in streams. They then move into the lake and some stocks may form a pelagic or off-shore migrating group and a benthic or in-shore migrating group ... The bull charr of the large lakes and rivers of the Pacific Northwest also live in streams for the first part of their lives and then move into lakes and begin feeding on other fish. These fish grow to a large size ... and sometimes conduct extensive migrations ...".

*Geographical Distribution*. Dolly Varden charr are distributed in western North America and eastern Asia. In North America they occur along the west coast from northern California, Oregon, Washington, through British Columbia, to coastal Alaska. Interior stocks, including bull charr, occur throughout the Pacific Northwest, western Alberta, through to the Yukon, southwestern Northwest Territories, and Alaska. In Asia they range from the Anadyr River in the north, southward through Kamchatka, the

Commander Islands, Sakhalin, Japan, to the Yalu River in Korea (Figure 27C) (Scott and Crossman 1973).

*Age and Size*. The general life span of Dolly Varden charr is 7-9 yr and some can become 18-20 yr old (Carlander 1953). Few, however, reach 10 yr of age. Sexual maturity is reached at age 3-6 for both the anadromous and freshwater populations. Males often mature a year earlier than females (Scott and Crossman 1973). Size of Dolly Varden varies greatly between locations and between freshwater and anadromous fish. Some anadromous populations only reach 2.5 kg and high-altitude and northern populations do not exceed a length of 30 cm. The northern form from Arctic slope drainages in Alaska seldom grows heavier than 3 kg. In Eva Creek, Alaska, anadromous 11-year-old fish were 43.5 cm long and weighed 0.47 kg. In Jasper National Park, British Columbia, freshwater Dolly Varden charr were 56-62 cm long (fork length) by year 7. Larger Dolly Varden or bull charr occur in the southern parts of its geographical distribution. A Dolly Varden charr of 102 cm and 14.5 kg in weight was captured in Idaho and individuals close to 13 kg have been reported from Kootenay Lake, British Columbia (Scott and Crossman 1973).

*Reproduction*. Dolly Varden and bull charr spawn in the fall in streams with gravel beds. They prefer to spawn in rivers with moderate currents and at temperatures near 7 to 8°C. Anadromous fish favour main channels of rivers with fairly strong currents and depths of 20 cm or more, or they choose springs. Anadromous fish enter rivers from May to December and generally migrate upstream during August to September. Populations living in large lakes move into inlet streams at about the same time. Some time after the anadromous charr have entered fresh water or before freshwater populations have arrived on the spawning grounds, the secondary sexual characteristics develop and changes in body shape, form, and colouration take place. Besides changes in colouration, the snout thickens and the lower jaw develops a pronounced kype, especially in anadromous males. Females do not change as much in colour and shape as males do during spawning time (Scott and Crossman 1973).

Non-anadromous Dolly Varden charr usually spawn every year, whereas anadromous fish in northern Alaska and lake-resident fish in Alaska and Kamchatka may spawn every second year or at undetermined frequencies (Armstrong and Morrow 1980). Spawning takes place from late August to late November. Most of the breeding activity of the northern form is in September and October and of the southern form during September, October, and November (Armstrong and Morrow 1980). Spawning primarily occurs during daylight. The spawning behaviour of Dolly Varden charr differs in a number of aspects from that described in the general section on Spawning Behaviour (*see* Leggett 1980).

Dolly Varden charr are aggressive fish and nesting areas are usually at least 6 m apart. Four to five males can attend a female during courting, but one is usually the dominant one. The kype and other secondary sexual characteristics return to the normal condition soon after spawning and the adults move down to the lake to spend the winter. Nests of Dolly Varden charr are 30.5-61 cm in diameter and are up to 30.5 cm deep (Scott and Crossman 1973). The eggs are buried at a depth of about 10 cm in the centre of the nest. Redds containing several egg pockets can be as long as 3.5 m and 1.2 wide (Armstrong and Morrow 1980).

Fecundity differs by area for anadromous and non-anadromous fish. Average number of eggs per gram of body weight was 4.1 for resident and 3.0 for anadromous charr. In general, egg size and fecundity increase with fish size (Armstrong and Morrow 1980). In Montana average egg counts range from 1,337 to 8,845 (bull charr), and in Alaska from 1,336 to 3,387 (Scott and Crossman 1973). Mature eggs are 3.5-6.0 mm in diameter and orange-red in colouration. In small freshwater residents in Alaska, fecundity can be as low as 42-346 eggs per female with eggs as small as 2.8 mm.

*Incubation and Emergence.* Eggs of Dolly Varden charr incubate in the gravel redds during the winter months. After about 4.5 months the alevins hatch in spring, usually from March to April, at a size of 15-20 mm. In rivers of the North Slope of Alaska incubation may last 7 or 8 months (Armstrong and Morrow 1980). Depending on environmental conditions especially temperature, the yolk sac can be absorbed after 18-70 days. Fry emerge from the gravel from April to June at a length of 20-25 mm. At first they remain inactive on the bottom, but start feeding after having filled their swim bladders with air to maintain neutral buoyancy.

*Juveniles and Immatures.* At first the juveniles of Dolly Varden charr remain in the stream of birth and appear to be sedentary. They are very aggressive and may establish feeding territories in the stream. The 1- and 2-year-old charr may leave the rearing areas in June and July and move to other parts of the river system. In streams that freeze over, Dolly Varden may move to springs to overwinter. In southern areas they are distributed through the whole stream during the winter months (Armstrong and Morrow 1980). They start to hide and burrow in debris on the bottom, when temperatures drop to 2-4°C.

Growth of fry during July and August is rapid and by mid-September most have attained lengths of 60 mm and weigh about 2 g. Parr in northern areas, e.g., Alaska, are 32-47 mm long in October. Yearly rate of growth of anadromous Dolly Varden charr is on average about 50-60 mm/year, with most growth occurring during the summer months. After they are about 6 or 7 yr old, growth rate slows down with increasing age. Stream-resident charr in Alaska may only grow 20-40 mm/yr and have life spans of 9-11 yr (Armstrong and Morrow 1980).

*Movements and Migrations.* Dolly Varden charr from the north coast of Alaska typically go to sea at 3 or 4 yr of age, after they have physiologically and morphologically changed into smolts. They lose their parr marks and become silvery. Females tend to change into smolts a year earlier than males. Dolly Varden smolt migration downstream to salt water occurs at night and begins when water temperatures have reached 3°C. This migration may start as early as May, but generally takes place during late June and July. Immature anadromous Dolly Varden charr do not make long migrations in the open ocean and generally stay close to shore near their home river mouth (Scott and Crossman 1973). They may stay in salt water from 60 to 160 days, moving only short distances from the river mouth, or they may stay in tidal waters. Migration back to fresh water can commence as early as late May or as late as early October, but usually takes place in mid-July to September (Scott and Crossman 1973; Armstrong and Morrow 1980). Anadromous populations of the southern form may stay 2 to 4 yr in streams and then move downstream as smolts from mid-May to early June. Non-anadromous populations may spend several months in streams and then distribute themselves throughout the river system or move into a lake.

As Dolly Varden charr become older and larger they can undertake extensive migrations. In southeastern Alaska they generally follow the coastline at sea within 6 m from the shore and stay close to the surface (Armstrong and Morrow 1980). They have been found to travel up to 350 km, including both fresh- and saltwater movements, and have been captured in the Bering Sea as far west as 170°W longitude (Armstrong and Morrow 1980).

Dolly Varden charr tend to return to fresh water in summer and autumn after several months in the ocean, and throughout Alaska many populations spend the winter in lakes. The mature charr enter fresh water first, followed by the immature fish (Armstrong and Morrow 1980). As in many other salmonids, Dolly Varden show strong homing tendencies and return to the stream of birth for spawning. When spawning is finished the adult fish move downstream to overwinter in springs and spring-fed regions of the river or possibly in estuaries. Non-spawners do not go to the spawning areas but overwinter elsewhere in the river system, in other streams, or in a lake (Armstrong and Morrow 1980).

### *Arctic Charr*

Of all the salmonids, the Arctic charr lives closest to the Arctic and occupies the most northerly waters of any other freshwater fish species. They occur as non-anadromous and anadromous forms and manage to live in harsh arctic environments due to special physiological adaptations and behaviour traits. Behnke (1984) recognizes three major centres of evolution in the Arctic charr complex: a European origin of the north and central European form and probably the eastern North American form; a Siberian origin for the vast and diverse groups occurring in northern Russia; and a Bering Sea area origin for populations in the Asiatic and North American river systems of the Chukotsk and Bering seas.

*Ecotypes*. Arctic charr of northern North America can be separated into a central-eastern Canada and southeastern Alaska group and a northwestern and northern Alaska group on the basis of mean number of gill rakers and pyloric caeca (McPhail 1961). Each group contains several forms of Arctic charr which can differ in depth distribution, size at maturity, time and place of spawning, existence of anadromous and non-anadromous stocks, colour, and morphology (Johnson 1984). Some forms occur sympatrically in the same lake.

In some alpine lakes there appear to be two basic forms: a large, predatory, fast-growing stock and a smaller-sized, slower-growing stock. These stocks differ in colour and morphometrics. In Windermere, U.K., two main stocks of Arctic charr exist. One stock spawns in autumn in shallow water (1-3 m) whereas the other is a deep-water (20-30 m) spring-spawning stock. These two stocks are very similar morphologically but they differ in time, place, and depth of spawning. In the Nauyuk Lake system, Northwest Territories, Canada, anadromous and non-migratory Arctic charr occur sympatrically in the same system. Both stocks spawn at the same time and no morphological differences could be established (Johnson 1980).

*Geographical Distribution*. Arctic charr occur naturally in lakes, rivers and inshore marine waters of northern North America, northern Asia, northern Europe, Iceland, Greenland and many Arctic islands (Figure 27D). In North America, Arctic charr are found in Newfoundland, Labrador, eastern Québec, and along the coast of the Atlantic Ocean north to Hudson Strait, Hudson Bay, the Arctic islands, Alaska, and the Aleutian Islands. In these areas they are generally restricted to within 100 km from the coast (Johnson 1984). Arctic charr that are distributed below 60°N latitude throughout Sweden, northern Russia, Finland, Great Britain and Japan are primarily non-anadromous. These stocks are land-locked and non-migratory. Some land-locked Arctic charr populations are found in the lakes of the European Alps and in the headwaters of the Lena and Angara rivers in the region of Lake Baikal. These charr occupy oligotrophic environments where, in Johnson's (1984) words, "only physiological and ecological generalists can survive by utilizing all possible resources". Arctic charr do not occur in the deeper lakes of central North America as in they do Europe. In North America this habitat is occupied by the lake charr (Johnson 1984).

*Age and Size*. Arctic charr generally grow slowly and live to a considerable age. They can reach full size at age 20 and can attain ages of 36 yr and older (Scott and Crossman 1973). Because predation is very light in northern conditions, many Arctic charr live out their life-spans. Age at first migration for anadromous populations ranges is about 2-3, 2-5, 5-7, and 8-9 yr for Arctic charr from the far east, Alaska, Northwest Territories, and some Arctic islands, respectively. Age at maturity, lengths and weights for these same populations were 4-11 yr, 30-45 cm and 0.84 kg for the far east; 6-10 yr, 45-60 cm and 0.9-2.0 kg for Alaska; 10-18 yr, 62-80 cm and 3.4 kg for the Northwest Territories; and 11-25 yr, 38-69.5 cm and 0.55-2.05 kg for charr from the Arctic islands

(Johnson 1980). Very large Arctic charr may reach a length of 96 cm and weigh 11-12 kg (Scott and Crossman 1973).

Non-migratory stocks become mature at a younger age and smaller size than anadromous charr. Sizes for freshwater populations can range from an average length of 16 cm for dwarf stocks to fish with maximum lengths of 74-86 cm, weighing 7-8 kg, for other stocks. In general, non-anadromous Arctic charr reach maturity at a mean length of about 30 cm or smaller and weigh about 1 kg (Johnson 1980).

*Reproduction*. Anadromous charr spawn in autumn in either lakes or rivers. Non-migratory charr may spawn in lakes in autumn and spring, but only during the autumn in rivers. In northern Canada, anadromous charr begin to move to the spawning grounds between late August and mid-September and spawn in September and October. Arctic charr from more southern regions may spawn as late as November and December (Johnson 1980).

Stream spawning is common in Labrador, western North America, Yukon Territory, and in rivers flowing into the Sea of Okhotsk. Spawning substrate ranges from coarse sand to gravel with boulders. Gravelly bottoms are preferred but sand bottoms are utilized when density of spawning fish is high or when gravel substrates are limited (Johnson 1980). Lake spawning is common in the central Canadian Arctic and in the Arctic islands. Spawning beds in lakes can vary in depth from 3 to 6 m. In European lakes Arctic charr have been found to spawn in depths varying from 1 to 100 m (Johnson 1980).

Arctic charr females may spawn every 2, 3, or 4 yr after reaching sexual maturity. Spawning frequency declines with age and size. A female Arctic charr that reaches sexual maturity at 10 yr of age and continues to spawn until up to 24 yr old may only spawn two or three times, and at the most four times (Armstrong 1984). In southern areas, female fish are known to spawn every year. Spawning in lakes takes place around low gravel islands with gravel of 3-10 cm in diameter and at depths of 2.3-5 m (range 0.2-11 m). Water temperatures in spawning areas vary from 2 to 7°C and current velocities range from 0.2 to 0.8 cm/sec (Armstrong 1984).

The spawning behaviour of Arctic charr has been described in detail by Fabricius (1953) and Fabricius and Gustafson (1954). Males arrive first on the spawning grounds in bright red spawning colouration and start to defend territories. Both frontal and lateral displays are shown in agonistic encounters. In the frontal display the fins are erected and the body flexed upwards from the vent. The bottom of the mouth is depressed, showing the intense black colouration of the interior that contrasts with the white or lemon-yellow of the lower jaw. Females arrive on the spawning grounds a few at a time, spawn, and depart. At first they rest on the bottom such that the red sides and belly are not shown. Females may be approached aggressively at first but soon the male starts courting her by swimming beside her between attacks. The female then commences to search for a nesting side by swimming low over the gravel with pectoral, pelvic, and anal fins scraping over the gravel substrate. When a proper site has been found she will start to dig the nest and is then usually attended by a male.

Females dig as many as 8 to 10 nests before all the eggs have been laid. The nests are roughly circular and 2-3 m in diameter. Before releasing the eggs the female crouches into the nest depression several times. Each time she crouches a male joins her. Actual spawning takes about 10 s. About one to five (Fabricius and Gustafson 1954) or four to seven (Frost 1965) spawnings can take place before the female will start to cover the eggs. She first performs slow snake-like sinuous movements which sweep the eggs into spaces between the gravel and cover them with small gravel. These egg sweeping and covering movements gradually change into normal digging motions. She moves slightly upstream of the nest and while making shallow, quick digging movements, moves gravel downstream over the eggs. Eggs can be buried under a layer of gravel 10-20 cm thick. This digging results in the construction of the subsequent nest in front of the first one. Spawning occurs primarily during the day. Females can release all the eggs over a period from 4 h to 3 d during which time she can prepare up to 8 nest pockets (Johnson 1980). She then continues to shape the redd and defends it for a few more days before leaving the spawning grounds. Redds can be 3.5 m in length and 1.2 m wide. Males abandon the female after she has laid all her eggs and search for other spawning opportunities. They may mate with several females during the spawning period.

The fecundity of anadromous Arctic charr varying in length from 38 to 60 cm ranges from 950 to 6,500 eggs per female or 140-377 eggs/100 g fish. Egg diameters range from 3.2 to 5.0 mm (Johnson 1980). In non-migratory stocks of Arctic charr ranging from 10 to 40 cm, fecundity varies from 21 to 3,100 eggs per female or 184-970 eggs/100 g of fish. Egg diameters for the non-anadromous stocks vary from 3.8 to 5.0 mm. Some populations that mature at small sizes (15-18 cm) only produce a small number of eggs but the eggs are relatively large (Johnson 1980).

*Incubation and Emergence.* In Windermere, U.K., incubation time of Arctic charr eggs decreases from 97 d at 4.0°C to 36 d at 12.0°C. Substantial mortality of eggs occurs at temperatures above 8°C. Soon after the yolk sac has been absorbed, which may take 2-3 months, the fry emerge from the gravel. They are about 20-25 mm long at that time (Johnson 1980). In Arctic areas, the eggs develop in the gravel over the winter at temperatures of about 0-4 °C. Temperatures above 7.8 °C are lethal. The alevins hatch in early to mid-April after an incubation period of 130 to 230 days, depending on the temperature. The fry emerge from the gravel after ice break-up in about mid-July (Johnson 1980).

*Juveniles and Immatures.* Soon after emergence the fry leave the breeding grounds. They are not aggressive at this time and do not defend feeding territories (Frost 1965). The fry stay close to the bottom at first, resting on their pectoral fins and hiding among stones. To feed, they dart to capture passing food particles and quickly return to their original station. In some lakes young fry immediately move towards the shore upon emerging and feed among the rocks in the littoral zone. In summer the juveniles may move to deeper parts of the lake or continue to occupy rocky shores and suitable sites in the stream (Johnson 1980). Fry and juveniles are generally benthic and change to a pelagic way of life as they grow larger, usually after about 3-4 yr. This shift in habitat coincides with a fading of the parr marks and the development of silvery sides, and with a change in behaviour to schooling.

Little information is available on the behaviour of Arctic charr between the end of the first summer and the time the first fish go to sea. Young Arctic charr may either stay in fresh water during their life cycle or move downstream to salt water for the first time in spring or summer when 4-5 yr old (range 2-9 yr and 10-20 cm in length). They tend to eat very little while in fresh water. Arctic charr that spawn in a lake stay in the lake during the winter and migrate to sea again in spring (Gyselman 1984). Johnson (1980) presumed that in Arctic lakes, "there is an annual migration of juveniles into streams and shallows in spring, followed by a retreat to deeper regions during winter."

The Arctic charr's ability to withstand low temperatures is remarkable. Temperatures of freshwater systems in the Arctic are generally between 1 and 2°C throughout winter and salt water temperatures during downstream migration in spring can be as low as $-1.5$°C. Although Arctic charr do occur in such low temperature conditions for various length of time, they tend to avoid them. Optimum temperature for growth rate of Arctic charr is between 12 and 16°C and the upper lethal temperature is 24°C (Johnson 1980).

Arctic charr generally spend relatively short periods in seawater and appear to be poorly adapted to marine environments. Anadromous Arctic charr are better able to live under saline conditions than non-migratory stocks. The non-anadromous stocks, however, are still able to osmoregulate although many have been isolated from the marine environment since the last glacial retreat 10,000 to 12,000 yr ago (Johnson 1980).

*Movements and Migrations*. Before reaching sexual maturity, young Arctic charr spend several years in fresh water followed by two to four seasons of migration to sea for feeding. When the juveniles reach the smolt stage in the spring of their fourth or fifth year, they migrate to sea annually until they reach maturity for the first time. In many Arctic freshwater systems the smolts are only able to migrate to salt water after ice and snow have melted in the stream and water starts to flow. The larger fish tend to move downstream first, followed by the smaller smolts.

In the ocean Arctic charr generally do not move far from the parent stream and disperse to feeding stations along the coast. Large charr travel farther than smaller ones. They tend to stay in shallow water (<3 m deep) and close to shore (within 45 m) (Moore 1975). They may move in schools composed of particular sizes in salt water. In some areas, such as in northern Norway, Arctic charr were found to travel long distances from their home stream. Although three-quarters of the fish were captured within 25 km of the stream of origin, some had travelled 100, 400, 500 and 940 km away (Johnson 1980). The period of feeding of Arctic charr in the ocean varies among different regions. It ranges from 35 to 56 d for the charr of Nauyuk Lake, and averages 40 d for Vardnes River charr. In several river systems Arctic charr tend to move up and down the stream throughout the summer. As fish from the local stream migrate out to sea, Arctic charr from other river systems may move in. These varied short-term movements may be adaptations to living in an uncertain environment (Johnson 1980).

Homing is relatively strong in Arctic charr and many return to the home stream for their first and subsequent spawnings. In many river systems the upstream migration begins in late July or early August, and peaks between mid-August and early September. The largest fish tend to enter the stream first (Johnson 1980). After returning to fresh water in autumn Arctic charr are quite inactive and rest on their fins on the river bottom.

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## Chapter 4

# BROODSTOCK MANAGEMENT

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## INTRODUCTION

Our salmonid resources are of immense value and present both recreational and financial opportunity for sustainable development. This fact explains society's intense interest in exploiting salmonid populations wherever they have been encountered. Ironically, these highly prized species have been so intensely abused as to eliminate them from much of their historical range (MacCrimmon and Gots 1979; Johansson 1981; Dunfield 1985; Nehlsen et al. 1991; de Groot 1992). Reasons for extinction of salmonid populations include: habitat loss and damage; inadequate passage and flows caused by hydropower; agriculture; logging and other developments; overfishing, primarily of weaker stocks in mixed-stock fisheries; and negative interactions with other fishes, including non-native hatchery salmon and steelhead (Nehlsen et al. 1991). Nelson and Soulé (1987) stated that efforts for "...maximizing current and future economic returns have been instrumental in the progressive decline in fisheries production worldwide". The "tragedy of the commons", characteristic of free-entry fisheries, must be abandoned in favour of a sustainable yield strategy for all of our world resources if harvest of natural resources is to generate the greatest value over the longest time.

Fortunately, science has provided much of the knowledge on which to develop long-term management plans in support of sustainable harvests. Throughout this chapter the term "management" is used to imply manipulations of species' performance characteristics and potential, both in terms of phenotype and genotype. In contrast to socio-political management of salmon (Argue et al. 1983), the term management is used in this chapter to imply consequences of significant impact on the biological performance of the species. This chapter deals with propagation of salmonids for both natural environments and for aquaculture.

The approach to management of wild salmon and trout populations often differs considerably from that for an aquaculture operation. However, it is paramount to both types of operations that there is a large enough complement of spawners suited to self-sustaining production of future generations. From this simple common starting point of broodstock requirements for both wild and artificially propagated salmonid populations, strategies for broodstock management diverge considerably according to the desired goal for the population in question. Brannon (1991) introduced the concept of a conservation hatchery and distinguished between such operations and commercial hatcheries. These distinctions will be highlighted in this chapter.

Several concepts that pertain to broodstock management are introduced in this chapter and will be developed in greater detail in other chapters. This is not intended as a "how to" guide on broodstock development and management because logistics appropriate to broodstock manipulations will vary with the specific goals of the endeavour and the site-specific opportunities available to the project. Rather, the goal of this chapter is to convey both the theoretical concerns and the practical tools that should be available to the manager of both wild and aquaculture broodstocks to enable sound decisions regarding direction of the broodstock program. This chapter develops the theme that effective broodstock management requires extensive consideration and priority setting of goals. Application of sound biological principles to founding and maintaining salmonid brood strains can be remarkably straightforward provided there is first a thorough definition of goals. Because broodstock programs must deal with generations, ad hoc broodstock programs are likely to be futile (Tave 1986). To this, Tave (1986) added that, as much as goals are necessary, well-conceived plans are mandatory.

## WILD VERSUS HATCHERY BROODSTOCK PROGRAMS

Managers of wild anadromous salmonid populations attempt to ascertain target egg deposition requirements for optimal seeding of natural spawning and rearing habitats. Actual egg deposition is assessed relative to this target. In the context of sustainable natural salmonid populations, the type, amount, and distribution of various habitats, together with nutrient characteristics of the watershed, are some of the factors that determine habitat carrying capacity for a salmonid stock. Sound management of natural salmonid stocks necessitates that strategies rely on quantification of life history performance. This must take into account highly variable life history pressures such as food availability, predation and competition. In the end, by controlling harvest pressure so that sufficient spawners escape exploitation and predation to meet target egg deposition requirements, the manager of natural populations relies on the fish themselves to select appropriate mates and generate offspring with survival potential. In contrast, the manager of a hatchery brood stock has no such respite even though there is likely to be much greater control over the selection of traits of the salmonid stock(s) of interest to the hatchery program. Through controlled diets, feeding schedules, and manipulated rearing conditions (i.e., fish density, water temperature and water chemistry), any given hatchery strategy and egg requirement can be met in terms of both the number and the size of spawners at the time of reproduction.

### *Choice of a Foundation Population*

To be successful, broodstock management strategies must be based on an appropriate foundation population that adequately represents the stock from which reproductive products will be obtained. Although there are no generally accepted definitions of the terms stock, strain, line or variety (Purdom 1993), the term "stock" is used as per Billingsley (1981) throughout this chapter to refer to an intraspecific group of randomly mating individuals with temporal or spatial integrity. However, in agreement with Spangler et al. (1981), it is not necessary to accept a single definition of a stock to communicate the implications of the concept for fisheries management. The term "strain" is used herein to represent a selected subset of a stock that could be used to develop several strains depending on the selection strategies applied to the founding population(s). As further illustration, there is a common trend in genetic variability among population subunits as follows: strain < stock < population < species. Also important is the quantitative determination of how much genetic variation exists initially in a salmonid stock, and the average phenotypic expression of this variability. This sort of quantification identifies the desirability of a potential foundation population for a particular management goal. Accurate assessment of variability in performance potential of a species should be made from data on several characters measured in different environments (Mallet et al. 1986).

Acquisition of brood stock is the most important step in management of a population (Tave 1986) and careful attention should be given to selection of founding populations (Hershberger et al. 1990*a*). Salmonid populations are naturally substructured into multiple genetically differentiated stocks that tend to be reproductively isolated from one another, both within and between watersheds. These stocks can be highly variable in their life history traits (Ståhl 1987). In fact, members of the taxonomic family Salmonidae are noted for their plasticity (Sterns and Crandall 1984; Saunders and Schom 1985; Thorpe 1986, 1989) in both life history characteristics (i.e., age at first maturation and fecundity) and morphometric characters such as body shape. It is this plasticity that a broodstock manager must consider in choosing the salmonid stock from which to extract brood or eggs. In light of the variability among salmonid stocks, and corresponding differences in potential of these stocks to achieve the goals of the management scheme being attempted, considerations for selection of a foundation population are presented in Table 1.

**TABLE 1**

Guidelines for choice of a salmonid foundation stock (Ingram 1988).

- Use local stock wherever possible, even if only relatively small numbers of brood stock are available requiring building-up in captivity. Beware of using too few individuals.
- Where no immediate local stock is available, choose one from an adjacent river system. It has been noted in transplant experiments that survival falls with distance between the donor and recipient sites.
- Where no stock is available from local sources or from nearby, choose a stock occupying a similar environment with respect to stream characteristics, water temperatures, presence of lakes on the river system, etc. For migratory species, similar coastal environments and orientation of coastline may be important.
- Where these criteria cannot be met, it is probably a sound strategy to obtain stock from as many sources as possible.
- Where non-local stocks are used, future brood stock should be taken from the survivors of earlier releases, rather than by bringing in new genetic material.
- Where very small numbers of local stock are available, it is possible to produce a hybrid between the local and donor stocks.

In addition to this wild stock orientation, Turner (1949) identified the following five criteria to govern introduction of a non-native stock:

- It should fill a need created by the absence of a similar desirable local species;
- It should not compete with valuable local species;
- It should not hybridize with local species;
- It should not be accompanied by enemies, parasites or diseases that might attack native species; and
- It should live and reproduce in equilibrium with its new environment.

### *Review of Current Practices and New Trends*

The goals of a program for hatchery propagation of salmonids to be planted into the wild are very different from the profit motive of an aquaculture program (Allendorf and Ryman 1987). As stated by Ferris and Berg (1987), sound management decisions require a thorough knowledge of the genetic and spatial discreteness of stocks. As well, an understanding of the impact of particular fishery practices on the genetic structure of a stock also is required. Indeed, it is difficult to discuss salmonid broodstock management without addressing at least some of the genetics issues discussed in detail elsewhere in this volume (*see* Chapter 11). Bailey (1989) noted that although management objectives for domestic and natural populations may be quite different, genetic principles are equally applicable to both. Just as it is important to choose the correct foundation population in support of the desired program of artificial propagation, it is important to consider the impact of salmonid brood removal on the wild spawning stock.

Tave (1986) and Kapuscinski and Jacobson (1987) discussed the concepts of genotype and phenotype. These concepts are particularly important to understanding the implications of spawning stock size and the difference between the genetic interpretation of actual spawning stock and effective spawning population. The terminology of these authors is as follows:

(1) genotype: a fish's genetic makeup; the entire set of genes carried by an individual.
(2) phenotype: the chemical or physical expression of a gene; the detectable properties (i.e., one or more traits) of an individual that are produced by the genotype and the environment.
(3) quantitative phenotype: a phenotypic character that is measured, e.g., weight.

Within these definitions, it is important to note that parents pass on genes and not their genotypes to their progeny. The process of sexual reproduction, in which offspring receive half of their genes from each parent, results in a genotype that is unique to the individual offspring and changes with each succeeding generation. The average effects of the parents' genes determine the mean genotypic value of their progeny (Falconer 1989).

Falconer (1989) clarified ideas about spawning populations stating that idealized conditions for subpopulations (i.e., a group of interbreeding individuals at a given location at a given time) are as follows:

(1) For each generation, there is a constant number of breeding individuals in all subpopulations;
(2) There are equal numbers of males and females of a dioecious species;
(3) All breeding individuals are replaced each generation;
(4) All mating occurs at random;
(5) All breeders produce a random number of offspring with an average family size of two offspring per breeding pair; and
(6) There is no migration, mutation, or selection.

It should be apparent that the term stock is used in this chapter in much the same context as Falconer's subpopulations.

Applying these general considerations for animal populations specifically to fish, Gall (1987) suggested that "a suitable reference population is one which is infinitely large and randomly mating with no selection, migration, or mutation occurring". Such a fish population will have five important characteristics as follows:

(1) Every male will have the same probability of mating with every female, regardless of genotype;
(2) For every mating, the number of offspring surviving to breed will follow the same probability distribution;
(3) Offspring surviving will represent a random sample of the genetic possibilities expected for the mating;
(4) Offspring will be equally viable, regardless of genotype; and
(5) There will be no selection among parents or offspring, so the probability of a mating between parents of particular genotypes will be a direct function of the frequency of each genotype.

Only after these conditions are met will the size of the brood stock actually equal the number of males plus the number of females. Where these conditions are not achieved, the effective population size is reduced and is calculated by the following formula (Falconer 1989):

$$N_e = \frac{4N_f\ (N_m)}{N_f + N_m} \tag{1}$$

where, $N_e$ = the effective population size, $N_f$ = the number of females in the spawning population, and, $N_m$ = the number of males in the spawning population.

The concept of effective population size will be used extensively in the following discussion. Maximizing $N_e$ (i.e., up to 685 per generation) is critical to the fate of a salmonid breeding program dedicated to enhancement of the wild resource. For the moment this statement will be left unsubstantiated. For aquaculture, in which survival under widely varying environmental conditions may not be of critical importance, maximizing $N_e$ may or may not be the appropriate management strategy.

## CHOICE OF GENETIC STOCK TO OPTIMIZE QUALITY OF OFFSPRING

Domestication is a virtual prerequisite of economically viable aquaculture. In contrast, considerable effort is required to prevent domestication in fish destined for stocking in the wild (Kapuscinski and Jacobson 1987). For a feral population undergoing enhancement, hatchery brood stock should be chosen randomly from the wild stock. For wild stock enhancement, the genetic composition of the particular stock should be changed as little as possible (Withler 1988). Hatchery fish derived from local stocks tend to perform better in their native environment than hatchery fish from other stocks. McIntyre (1991) indicated that selection is counterproductive to production of fish for release into the wild. There seems to be no justification for intentional breeding in stocking programs.

A predominant theme of resource enhancement is conservation of the complex natural stock structure of each species. A stock enhancement program should not be attempted without careful consideration of how the enhancement effort can be structured. Release of salmonids to the wild should avoid competition between the fish being stocked and the natural populations with which they must interact (Allendorf et al. 1987). In the wild, fish that avoid predation and compete for available food and space are those that tend to survive (Bailey 1989). Fish that survive tend to have the greatest genetic variability. Wide genetic variation within a natural stock assures that its members have characteristics that confer survival potential under changing environmental conditions. Changes in environment can result in significant changes to a predominant phenotype (McIntyre 1989). For a stock with little genetic variability, harsh environmental conditions, such as periodic diseases, can result in extinction.

It is the genetic generalists of a stock that are able to tolerate the widest variety of environmental conditions. This wide genetic diversity increases the probability that those fish with advantageous combinations of genes will be produced in sufficient numbers to survive to spawn (Bailey 1989). Rasmuson (1981) suggested that the adaptive strategy of a species or population can be seen as the way its members cope with the prevailing situation. Rasmuson (1981) identified the following factors as important:

(1) abiotic factors in the environment, such as temperature and annual cycles of day length;
(2) interaction and competition with other species in the ecosystem; and
(3) the genetic potentialities of the population, which are dependent on its origin and previous history.

Principles of genetic variability impose critical limitations on wild stock management. The long-term implications of management of natural populations, in ignorance of their adaptive gene complexes, are losses of genetic diversity and, most likely, productivity (Altukhov and Salmenkova 1987). Tave (1986) cautioned that, "...when you do not know what you want to do, or you do not know what phenotype is the best, no selection is the most appropriate form of selection for populations of game fish. Unintentional, unplanned, random changes usually do not improve productivity.". Programs that produce fish for enhancement of wild stocks should not attempt to alter the stock's genetic constitution to perform well under hatchery conditions. Brannon (1993) stated that problems attributed to hatchery fish are not the fault of the hatchery concept, but rather the fault of how hatcheries have been managed. It is important to attempt to conform to and work within the ecological limitations of the species. This includes its behavioural repertoire and the genetic variation of the foundation population (Allendorf and Ryman 1987).

Means towards this end are reflected in advice presented to the American Fisheries Society (Paul Brouha, AFS, Bethesda, MD, pers. comm.) at a 1994 workshop on the uses of cultured fishes in fisheries management: (1) Brood stock for wild stock enhancement should not be retained under hatchery conditions from year to year, but rather taken each year from the natural breeding population; (2) Hatchery programs for conservation of wild stocks should minimize the amount of time fish are retained prior to release to avoid adaptation to artificial hatchery environments; (3) Fish stocking should be pursued within an ecological framework that integrates the relative importance of predation, competition, and abiotic factors for all life stages; and (4) Releases should be limited to levels that will produce an escapement equal to 50% of the natural spawning target. This advice is theoretically sound but clearly begs the issue of the desperate status of some stocks. This question will be addressed later in this chapter.

### *Sampling a Wild Stock to Ensure Maintenance of Genetic Characteristics*

The genetic goals of aquaculture of confined salmonids, and those pertinent to raising fish for release to wild populations, cannot be achieved simultaneously with the same stock (Allendorf and Ryman 1987). Genetic principles must be considered when determining the consequences of brood extraction on the fate of the wild stock. Populations with narrow gene pools are successful only in stable environments or in the absence of competition. Such conditions are the normal realm of aquaculture but there are limits here as well. Marine net-pens are a largely uncontrolled means of salmonid confinement. Major year-to-year fluctuations in marine conditions may also require the genetic capacity for a flexible stock response to a changing environment in marine cages.

Natural stocks of salmonids do not often meet the idealized conditions for population reproduction identified above (Tave 1986; Falconer 1989). The most notable departures from such ideal conditions are the number of spawners among years, especially under conditions of heavy exploitation, and unequal numbers of males and females. The greater the departure from these idealized conditions, the greater is the probability that spawning pairs will have common ancestors (i.e., within the recent past) and hence contribute to inbreeding within the spawning stock.

Having introduced the concept of effective population size above, $N_e$ is now used to describe an inbreeding coefficient as $\Delta F$ ($\Delta F = 1/(2N_e)$; Nelson and Soulé 1987). This coefficient, expressed as a percent, is an average value for the brood stock used to produce the new generation; it is a measure of the increase in inbreeding produced by a single generation of mating in a closed population. Note that this coefficient always represents a reduction in genetic variability. It is in essence a penalty to the broodstock program for not working with an infinitely-large, randomly-mating stock. As long as a manager works within a particular, finite brood stock, the best $N_e$ that can be attained is that of the foundation population. As an example, if 300 females and 100 males were extracted from a wild population, the $N_e$ of the brood stock would be 300 ($\Delta F = 0.17\%$). While this value is low, it still represents an increase in average homozygocity of the breeding stock. Even if a subsequent ($F_1$) brood stock of 300 females and 300 males were used, the $N_e$ could be no more than that of the foundation population. In contrast, if 50 females and 50 males were developed from the progeny of the original brood stock, $N_e$ of this group would be 100 ($\Delta F = 0.5\%$). This illustrates an increasing level of inbreeding. It should be noted also that $N_e$ (and therefore $\Delta F$) has little meaning relative to purposely inbred lines.

Cross and King (1983) indicated that inbreeding increases most rapidly under conditions of disproportionate sex ratio. While it is wise for the manager of a wild brood sampling program to have equal numbers of males and females in an attempt to maximize $N_e$ of the hatchery stock (Thorgaard and Allen 1987), an equal number of male and female salmonids in a wild stock is the exception rather than the rule. Purdom (1993) stated that females often live longer than males and therefore represent increasing proportions of the older fish in stocks that spawn more than once. As well, factors such as precocious maturation among male salmonid parr in wild populations, which tends to increase mortality among male parr, often result in a heavily female-biased spawning stock (Dalley et al. 1983). Atlantic salmon (*Salmo salar*) grilse stocks (i.e., those that return to spawn after only one winter in the sea), indeed most of the salmon stocks of Newfoundland, Canada, have a sex ratio in the order of three females per male (O'Connell and Dempson 1991*a*). Occasionally this ratio is as high as 16:1 (O'Connell and Dempson 1991*b*). Except in heavily managed hatchery stocks, the effects of a disproportionate sex ratio and survival differences among families may reduce $N_e$ by an order of magnitude or more (Nelson and Soulé 1987), thereby greatly increasing $\Delta F$. The means by which natural stocks maintain such biased sex ratios as a stable evolutionary strategy will be discussed later in this chapter.

Under conditions of disproportionate sex ratio among natural spawning stocks, removal of equal numbers of males and females in support of a hatchery program will further bias the sex ratio of the remaining natural spawners. The consequence may be an effective population size much smaller than that required to maintain the stock. Frankel and Soulé (1981) indicated that in normally outbred species, a 10% increase in the inbreeding coefficient may result in a 5-10% decrease in fitness for a particular reproductive trait and a total decrease in reproductive performance of 25% or more. This illustrates the need for careful attention to hatchery practices. Franklin (1980) considered that, for an isolated native stock to maintain its historical level of genetic variation and thus retain its long-term adaptive potential, minimum $N_e$ would have to be on the order of 500. Tave (1986) indicated that an effective population size of between

68 and 344 per generation is sufficient for stocks that will be used in food fish and bait fish farming. Depending on the goals, between 424 and 685 per generation are required for stocks that will be released to natural bodies of water.

Any removal of potential spawners from a natural stock will impact on the target egg deposition for the year in which brood are removed. However, any extraction of brood fish that further biases the sex ratio of the remaining stock may have long-term consequences that will persist well beyond the initial extraction. Likewise, for the manager of wild salmonid stocks, those that have declined to levels significantly below the minimum $N_e$ recommended for wild populations may be experiencing a genetic bottleneck. In cases where a stock is at such a low level that guidelines for the uses of cultured fishes in fisheries management cannot be met, some compromise will be necessary. The question becomes one of whether it is better to do something or to do nothing. It is apparent from growing criticism of past fish stocking programs (Evans and Willox 1991; Hilborn 1992; Meffe 1992; Brannon 1993; Stickney 1994) that ill-conceived schemes may have consequences potentially more serious than doing nothing.

Where stocks are low, the first approach should be to question why. Field studies are needed to verify the ecological parameters of natural populations. A possible sequence of endeavour toward stock rebuilding might be: harvest controls and fishery regulations; habitat evaluation and rehabilitation; and, once these actions have provided the means to support population recovery, design and implementation of a stocking program.

There are many recommendations within the fish culture literature on values of effective population size in various situations. In fact, there is no single value of $N_e$ that is appropriate for all stocks (Kapuscinski and Lannan 1986). This is due to the fact that there is as yet no satisfactory means to determine the minimum amount of genetic variation required for populations to persist in unpredictable natural environments (Kapuscinski and Jacobson 1987). This constitutes another consideration regarding choice of a wild stock as a potential foundation population.

In situations where a natural stock is too depleted to provide adequate numbers of spawners to support a breeding program, there may be little choice but to extract some of the stock (i.e., perhaps 10%), supplement this brood stock with completely randomly chosen spawners from a nearby stock, as per the guidelines of Table 1, incubate the eggs in a reliable quarantine incubation facility, and return the fry to the watershed that is experiencing stock collapse. Where natural stocks of native salmonids are insufficient to provide spawners to a hatchery program, it may be necessary to collect gametes from several wild stocks and then cross-breed these stocks in an attempt to obtain adequate numbers of juveniles with sufficient genetic variability to encourage survival under stocking conditions (Krueger et al. 1981; Brannon 1991). This protocol would outbreed the stock(s). Where stocks can be obtained from the same geographic area, outbreeding should minimize the risk of disrupting coadapted gene complexes (Falconer 1989) critical to survival of the stock. This strategy draws on the natural phenomenon of straying of migrating salmonids among watersheds that serves to maintain some gene flow between natural anadromous salmonid stocks (Quinn et al. 1991; Gall et al. 1992; Labelle 1992; de Groot et al. 1994). Once gametes have been obtained, implementation of any hatchery stocking program should undertake to minimize inadvertent hatchery selection on the stock to be released back to the wild. Means of improving effective population size of a limited brood stock will be discussed later in this chapter.

### *Sampling Wild Stock to Ensure Adequate Genetic Variability for Future Breeding Programs*

Principles of population genetics become important when it is necessary to maintain stocks by artificial propagation. Gall (1987) indicated that when mates are more closely related to each other than individuals chosen at random, the mating is referred to as inbreeding. Generally, the greater the inbreeding, the more pronounced the depression in performance of specific traits (Tave 1986). Traits most commonly noted to exhibit performance erosion as a result of inbreeding include: increased fry abnormalities; reduced survival; reduced growth rate; and, lowered reproductive success (Aulstad and Kittlesen 1971; Gall 1983; Kincaid 1983; Fredeen 1986). Kincaid (1976*a*, 1976*b*) showed that a 25% loss of genetic variability in rainbow trout (*Oncorhynchus mykiss*) due to inbreeding was associated with an increase in morphological deformities (38%), decreased food conversion efficiency (6%), decreased fry survival (19%), and decreased weight at 147 days (11%) and 364 days (23%). Harmful effects of loss of genetic variation in small stocks are well documented in fish and a variety of other organisms (Ralls and Ballou 1983).

Studies with a variety of agricultural species have shown that even a 10% loss of genetic variation has detectable harmful effects on such important traits as survival and growth rate (Falconer 1989). In aquaculture, such losses in productivity are costly. For enhancement of wild stocks, such losses can have serious implications for continuation of the stock. Clearly, steps must be taken to deter such breeding sources of inferior performance. Withler (1988) suggested that a valid hatchery objective is to maximize retention of genetic variation present in the founding population. This approach requires knowledge of factors that influence genetic variation and how to quantify variation within and among stocks.

Brannon (1991) suggested that managing a hatchery brood stock without influencing its gene pool is probably impossible. Having once recognized that hatchery practices can have serious impacts on the future performance of salmonid stocks, it is important to understand how these impacts come about. By convention, all selection pressures imposed on fish populations by the environment are considered to be natural selection, even though the particular environment may have been artificially created or altered by human activities (Bailey 1989). Hatchery environments impose the need for human intervention in all aspects of the salmonid's life-cycle. Such intervention necessitates careful consideration of potential consequences of hatchery practices. Detrimental effects due to culture in an artificial environment, such as domestication and inadvertent selection, accumulate with time.

Ståhl (1981) noted that approaches to avoid inbreeding fall into three general categories: (1) use of large, random mating populations; (2) use of systematic line-crossing schemes to eliminate mating of close relatives; and (3) strain crossing to produce hybrid populations. As selection programs and mating schemes are covered in detail in Chapter 11, this chapter is limited to concerns about inadvertent selection and to hatchery procedures that are used to propagate the hatchery stock.

Inadvertent selection takes place when spawners that are mated are not a random and representative sample of the foundation population. Bailey (1989) stated that inadvertent selection also occurs when traits are correlated or associated with one another. When a genetic relation exists between two traits, selection programs designed to alter one trait may impact on other genetically correlated traits. Traits that are related to the fitness of the organism are especially significant (Kapuscinski and Jacobson 1987). An example is provided by Ehlinger (1977) who found that increased resistance to furunculosis in trout was associated with susceptibility to gill disease. This form of inadvertent selection is of particular concern in salmonid management strategies for wild stocks, especially those in which user groups advocate selection for life-history traits that are not well understood. One such example is angler-group pressure for programs that favour multi-sea-year Atlantic salmon over grilse. Amid the confusion that exists over the genetics of this issue (Gall 1983; Wilkins and Gosling 1983; Meerburg 1986), it is questionable if a broodstock program could be designed without imposing some risk to stock fitness.

Improperly designed selection programs and poor hatchery practices reduce $N_e$ and encourage inbreeding and loss of genetic diversity (Kapuscinski and Jacobson 1987). As hatchery practices invariably result in a $\Delta F$ that accumulates from one generation to the next (Bailey 1989), steps must be taken to minimize this progression. Hershberger et al. (1990*b*) recommended that factors that influence inbreeding must be integrated into the design of any program to develop genetically improved aquacultural stocks.

Consideration of $N_e$ and $\Delta F$ is further complicated by individual differences in fertility among potential spawners. As noted by Withler (1988), common hatchery practice is for the milt of several males to be applied sequentially to egg batches during stripping and fertilization. Another common practice is for the milt of several males to be pooled and applied simultaneously to one or more egg batches before addition of water. These practices are clearly inadvisable as there are large differences among the capabilities of males to fertilize eggs (Gharrett and Shirley 1985). Withler (1988) showed that the capacity of sperm from individual males to fertilize eggs can vary during the spawning season. Also, sperm of some males can result in good fertilization when applied alone but, when applied in a pooled milt mixture, do not result in fertilization in proportion to the amount of sperm from the individual male that was used in the milt pool (Withler 1988).

These results are similar to those of Forester (1965) and Gharrett and Shirley (1985), and confirm that a reduced $N_e$ will be the consequence of differences in fertility or potency among individuals. To this must be added the consequence of "superoptimal" stimuli (Tinbergen 1948). In hatchery operations that do not use family-oriented breeding schemes (*see* Chapter 11), and where hatchery personnel must select breeding pairs solely on the basis of phenotype (i.e., assortative mating; Gall 1987), there is a strong tendency to mate the largest of the males with the largest of the females. Effective population size will be reduced further if the spawning process favours success of gametes from some individuals (i.e., those deemed desirable) over those of others (Hill 1979). As the normal spawning period lasts from several days to several weeks, depending on species, it is likely that hatchery males are not equally mature at any given time and, therefore, unlikely that they have the same fertilization capabilities on any given day. In favour of genetic conservation, Gunnes and Gjedrem (1978) recommended single pair mating with family lots held separately until hatching.

A considerable amount of research has been done to develop breeding schemes to maximize $N_e$. Unfortunately, many hatchery practices designed to minimize costs and facilitate operations have been shown to reduce $N_e$. Pooling of semen is one example. Nelson and Soulé (1987) pointed out that natural spawning can result in as many as 25 males contributing to fertilization of one female's eggs. They stated that the long-term result of some hatchery practices is irreversible loss of genetic variation. The simple hierarchial mating system used at some hatcheries (i.e., mating each male to three different females) does not compare with the genetic variability inherent in natural spawning. To prevent unintentional selection in situations where family origin can not be determined, Tave (1986) suggested the following precautions:

(1) Spawn fish over the entire spawning season;
(2) Spawn fish of all sizes;
(3) Spawn as many fish as possible (you only need to keep a fraction of each spawn); and
(4) Do not cull the slow growers or those with poor secondary sexual characteristics.

To this should be added the precaution that some form of factorial mating system be applied. This will force a conscious effort to structure egg stripping and fertilization activities based on predetermined goals rather than on convenience.

The hatchery manager must be very clear about the goals of the operation. In fact, it may be necessary to have at least two parallel strategies. One strategy might cater to commercial production requirements and therefore demand hatchery practices that maximize financial benefits per brood fish. A parallel strategy might be implemented simply to support the hatchery brood line. The fertilization strategy adopted at any given hatchery will depend on the type of mating program (e.g., full-sib, half-sib, random; *see* Chapter 11) and the selection goals. Potential variability in spermatozoa quality among male salmonids suggests the wisdom of some form of factorial mating system using single-pair matings. An example of such an approach is to take eggs from each of three females, divide them into three portions, and fertilize these portions individually with sperm from three different males, thereby resulting in nine sib-groups (Gunnes and Gjedrem 1978).

Eventual loss of genetic variation from finite hatchery stocks is inevitable. Unfortunately, structured breeding programs that strive to maximize genetic gain for specific characters can be very expensive (Figure 1), both in terms of hatchery space and the logistics of hatchery operations. Withler and Beacham (1994) provide both a means to address this quandary and the answer to how salmonid populations with highly female-biased sex ratios maintain their genetic diversity. Withler and Beacham (1994) indicated that $N_e$ can be greatly improved if multiple males each fertilize equal portions of eggs from multiple females. In the wild, salmonid spawning behaviour is promiscuous. Females may spawn several times with different males. This behaviour helps to overcome the direct limitations implied by the concept of effective breeding population. In light of her earlier caution about pooled semen (Withler 1988), Withler and Beacham (1994) noted that potency of males contributing to a semen mixture was more equal when the pooled semen was held for an hour prior to use than when fertilizations were conducted immediately after pooling of the milt. Their conclusion was that holding semen for at least an hour before use would tend to equalize male contributions. They suggested that addition of semen from one male to the eggs of one female, with subsequent addition of semen from one or more males as 'insurance' against an infertile male, satisfactorily mimics single pair matings in a hatchery operation.

In light of these observations, if separate family lines cannot be maintained and the hatchery manager must limit the range of breeding goals to just maintaining stock performance, Withler and Beacham (1994) recommended that "... addition of semen from single males to egg batches pooled from several females can be used to maintain genetic variation within hatcheries. One possibility is to subdivide the eggs from individual females and then pool them to form replicate egg batches, each containing some eggs from all contributing females. The fertilization of each egg batch with semen from a different male will prevent the loss of all the gametes from a single female in the case of an infertile male, and will provide some degree of factorial mating without a requirement for semen pooling.". Such an approach is illustrated in Figure 2, where eggs are combined from groups of three randomly selected females. They are then subdivided into three roughly equal lots, and the subsets fertilized, either by a single male or with overlapping pairs of males if the size of the brood stock is seriously limited. McIntyre (1991), while acknowledging that sperm may compete for eggs thereby making the use of pooled milt inadvisable, cautioned that use of a single infertile male will waste available eggs and therefore loss of economic potential. The mating scheme of Figure

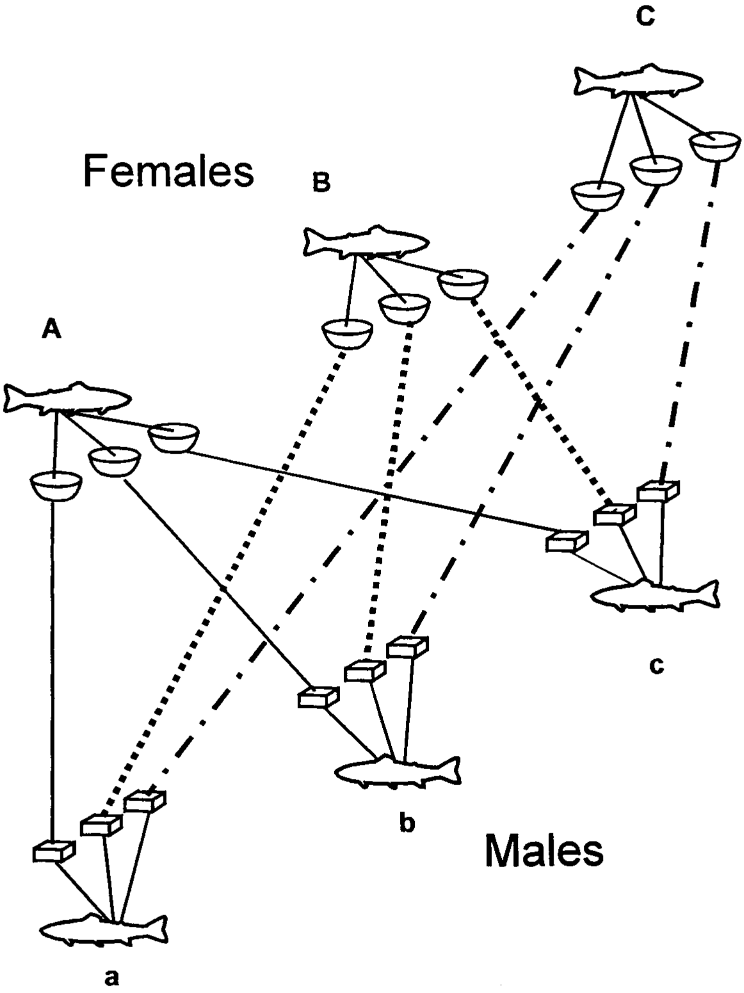

FIGURE 1. Family selection types of breeding programs can be logistically demanding.

2 is designed in consideration of Withler and Beacham (1994) to pursue a 1:1 sex ratio, yet mimic wild spawning in which different males may contribute genetically to the eggs of a single female and to eggs of different females.

Within this scheme, the three subsets of eggs, once adequately mixed, are statistically homogeneous. Preference for single-pair matings is indicated in Figure 2 by the solid mating lines. The dashed mating lines are used to convey that overlapping matings using two males could be used in situations where the consequences of a single inviable male could constitute an unacceptable loss of eggs as indicated by McIntyre (1991).

Where unacceptable consequences of male infertility dictate use of pairs of males with each egg lot, batches of pooled sperm should be held under controlled conditions for at least one hour. Some sperm from males a and b is added to two different areas of container A. All of the gametes are then mixed before fresh water is added to activate the spermatozoa. Sperm from males b and c fertilize ova from group B, and males c and a fertilize ova from group C. While this procedure encourages both maximum fertilization and continued economic return on investment, it does not lend itself to pedigree programs in which future spawners must be chosen on the basis of family lines rather than solely on phenotypes. Withler (1988) indicated that a valid hatchery objective is to maximize retention of genetic variation present in the founding population. Again the goals of the particular operation must be considered carefully. Under the breeding scheme of Figure 1, some egg mortality due to sperm infertility may be an essential overhead to the hatchery operation.

Allendorf and Ryman (1987) stated that approximately 10% of genetic variation will be lost in a stock with an $N_e$ of 50 after ten generations. Although those authors suggest that a 10% contribution of wild fish every second or third generation usually would be sufficient, procurement of additional brood stock for such outbreeding is potentially costly and presents more concerns about disease. Alternative approaches are implied by Gall (1987), who stated that mating of inbred but unrelated parents results in a non-inbred offspring. Nelson and Soulé (1987) indicated that genetic variation, introduced by even a single migrant per generation, may swamp the effects of inbreeding regardless of the size of $N_e$. For an aquaculture operation, incorporation of a disease-free breeding line from another hatchery may be the least costly approach. For wild stock enhancement, there likely will be little choice but to go back to the wild stock.

Regardless of the nature of the operation, a hatchery manager should aspire to quantitative decision criteria by which to determine when to supplement a given brood stock. Even after-the-fact indicators of genetic deterioration, such as increasing fry mortality and deformities, do not provide absolute evidence of stock deterioration. Such events can be an artifact of culture conditions. Accordingly, a monitoring system should be established for each strain in order to determine the success of the program in

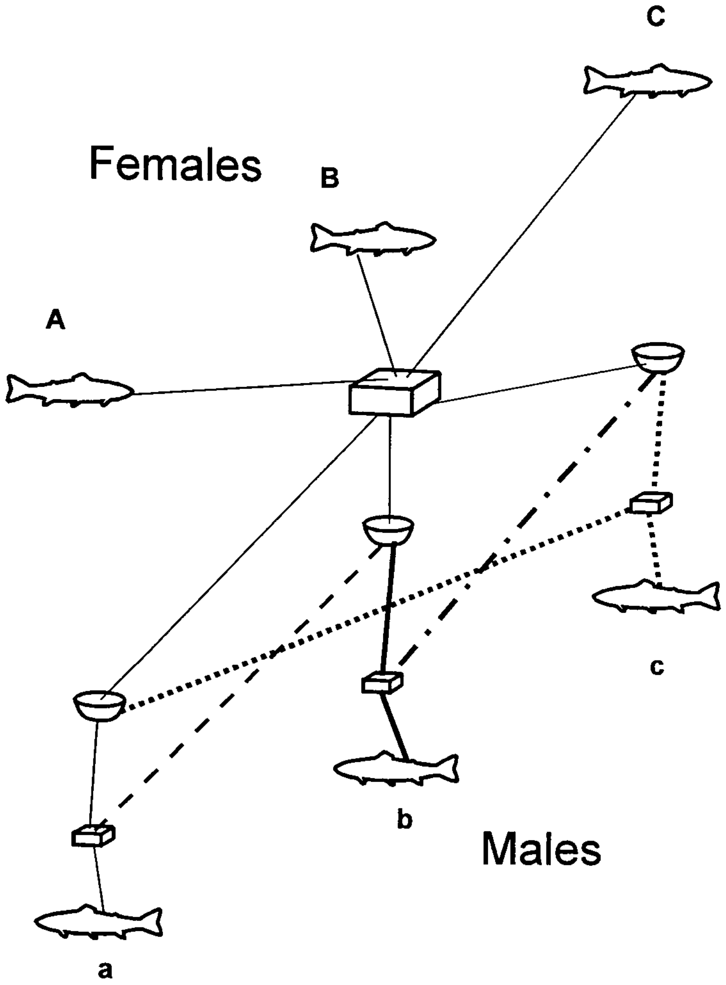

FIGURE 2. Cost factors typically limit hatchery programs to simple breeding plans and "overlapping" male spawners to avoid fertilization failure due to poor sperm quality.

maintaining genetic quality of the resource (Ryman 1981) rather than just assuming that the hatchery program is adequate for the goals of the operation. This requires actual measurement of the pattern of genetic variation.

Allendorf and Phelps (1981) advised that the pattern of genetic variation in a species should be measured in terms that allow description of at least four different measures of diversity:

(1) the amount of genetic variation in the average population;
(2) the variability in amounts of genetic variation in different populations;
(3) the amount of genetic differentiation between populations; and
(4) the pattern of genetic similarity among populations.

This level of quantification of genetic variability is not practical for many hatchery operations. However, sampling of hatchery stocks to obtain material for analysis of genetic profile is becoming more common, especially among hatchery programs that strive to develop patented lines for sale to other aquaculture businesses.

Actual genetic analysis of samples procured from potential brood fish requires specialized knowledge and equipment that is beyond the range of the average hatchery operation. However, such services often are available, albeit for a price, at universities, some technical institutes, and in the private sector. Other than to introduce the terms "genetic fingerprinting", mitochondrial and nuclear DNA, and protein electrophoresis, further discussion of this issue is deferred to Chapter 11.

### *Sampling Wild Salmonids to Select Best Stocks for Domestication*

According to McAuley (1981), there are two main reasons for developing a domestic brood stock: (1) to provide a stock of salmonids specifically suited for survival and growth under the culture conditions specific to the operation; and, (2) to establish a secure and reliable source of eggs. Selection brings this about via a breeding program in which individuals or families are chosen in an effort to change the expression of the mean of the desired trait in the next generation of the stock undergoing selection (Tave 1986). If different selection strategies are applied to the same foundation population, the resulting progeny from each distinct strategy will form the basis of different hatchery strains. Doyle and Talbot (1986) stated that domestication selection is indirect selection that is an incidental byproduct of grow-out and broodstock management practiced in genetically closed stocks. Identification of the likely sources of variation that occur in salmonid populations can be simplified (Tave 1986); phenotypic variance ($V_P$), which is observed for a quantitative trait, is the sum of the genetic variance ($V_G$), the environmental variance ($V_E$), and the interaction that exists between the two ($V_{G-E}$). This provides the following relationship:

$$V_P=V_G+V_E+V_{G\text{-}E} \tag{2}$$

While $V_G$ may be further divided into its components, Equation 2 provides sufficient elucidation of phenotypic variance to set the stage for Iwamoto et al. (1986) who indicated that strains can be characterized by the magnitude of genotype-environment interactions. General purpose stocks perform moderately well under a range of culture conditions. Special purpose brood stocks have desirable traits for specific conditions.

A breeding program aimed at producing fast-growing fish with high food conversion and docile behaviour for aquaculture should start from a foundation population containing a large amount of genetic variability (Allendorf and Ryman 1987). Identification of a foundation population requires that the enterprise do the following: (1) explicitly develop specific, quantitatively-defined goals; (2) conduct a survey of potential candidate stocks; (3) develop a baseline inventory of stock characteristics, including disease profiles; and, only after resulting data have been analyzed, (4) adopt a plan for collection of brood stock.

For an aquaculture operation with access to commercial sources of eggs, this baseline work may entail little more than correspondence with potential suppliers. If commercial supplies of eggs are not available or are unacceptable to the goals of the endeavour, implementation of a salmonid program may entail considerable expense to sample and subsequently choose among wild stocks. The second and third steps identified above require that data be gathered (or used from available sources) to quantify phenotypic characters of significance to the endeavour. It is especially important to provide information about the quantitative characteristics of the stock. Included should be considerations of disease potential, growth rate, survival, yield, food conversion, fecundity, and measures of the mean, variance, standard deviation (SD), coefficient of variation (CV), range, and heritability (i.e., the relative importance of heredity in determining phenotypic values) of the characters of interest.

Withler et al. (1987) cautioned that choice of stocks for aquaculture should not be based simply on the phenotypic characteristics of natural populations. Srb et al. (1965) indicated that economic characters of animals are largely the result of complicated interactions among genes, and selection on the basis of phenotype alone is likely to make mistakes from a genetic point of view. Multiple genes in the same organism may affect the same character. Good estimates of phenotypic and genetic characteristics are needed for planning a selection scheme (Gjerde and Gjedrem 1984). Accordingly, the concept of heritability, defined by Falconer (1989) as the degree of resemblance between relatives, is introduced:

$$h^2=V_A \;/\; V_P \tag{3}$$

where, $V_A$ = the additive variance component; and $V_P$ = the total phenotypic variance.

This ratio, called heritability (i.e., $h^2$), defines the relative importance of heredity in determining phenotypic values that can be anticipated in offspring of the next generation. Heritability expresses the extent to which phenotypes are determined by the genes transmitted from the parents. This value determines the degree of resemblance between relatives and is therefore of importance in breeding programs. While the concept of total phenotypic variance (i.e., $V_P$) is relatively easy to grasp as the overall variability of characters encountered among progeny of a given mating, $V_A$ may require further discussion for those with little background in quantitative genetics.

Breeding experiments have suggested that multiple genes in the same organism may affect the same character (Srb et al. 1965). Many aspects of quantitative inheritance may be accounted for on the basis of the way in which genes are expressed. This may be in the form of: 1) dominance, in which the genetic component of a phenotypic trait from one parent may override the contribution from the other parent; 2) addition, in which the effects exerted by different genes acting in concert produce an effect (i.e., a metric character) in the progeny, the magnitude of which is a result of the sum of the effects of the individual genes; and, 3) epistasis, in which a gene or genes may interact to control the expression of other genes.

Genes that show no dominance are sometimes called additive genes. The additive variance is the main reason for resemblance between relatives. It is the only aspect of genetic variability that can be practically estimated from observations made on the population. Additive genetic variation is an exploitable genetic resource for stock improvement. In most cases, selection is the most effective method of genetic improvement available to fish breeders (Gall 1983). The ratio $V_A/V_P$ expresses the extent to which phenotypes are determined by the genes transmitted from the parents.

The concept of heritability is extremely important in breeding programs and refers to expected gains in expression of a phenotypic character in the next generation based on the magnitude of differences within the parent stock among different families (Gall 1983). In a breeding population, this expectation of differences is the basis of selection potential; the larger the $h^2$, the greater the potential for selection gains. It should be noted here that selection changes gene frequencies. Therefore, $h^2$ must change at each generation of selection (Roff 1992). The theoretical basis for breeding programs is well illustrated graphically by Srb et al. (1965, Figures 14-16 and 14-17).

Some traits, such as those associated with reproduction, have low heritability (Kirpichnikov 1981) and therefore are not good candidates for a breeding program. Fortunately, many of the traits of interest to a broodstock manager offer greater potential. Growth often is considered to be a desirable trait for genetic improvement (Gjedrem 1983; Mahon 1983; Gjerde 1986; Hershberger et al. 1990*b*). As most of the variation in growth is genetic (Gunnes and Gjedrem 1978), it is important to select the best strains as potential candidates for the foundation population for future selection programs. This example of variability in growth performance among salmonid stocks, and the potential for gains in performance of this trait, illustrates how a broodstock manager can use the essential raw material of a wild stock to build a broodstock management program.

Gjedrem (1979) achieved growth gains of 30% per generation. However, it should be noted that growth potential of salmonid samples in a baseline survey does not equate to observed fish size. The growth performance of individuals in a wild stock must be interpreted by examination of fish scale patterns of individual specimens relative to the overall relation between fish scale radius and fish size for all of the fish sampled. This method of back calculation of length at a given age provides a history of individual fish growth performance (Bagenal and Tesch 1978; *see* Chapter 2).

An example of a typical breeding program's goals and plans is provided by Gall (1979). The explicit goal of that specific endeavour with rainbow trout was to improve egg size, number of eggs/kg, hatchability, and weight at one year. Another consideration in broodstock logistics is the practicality of the endeavour. Iwamoto et al. (1990), working on improving the level of flesh colouration in coho salmon (*Oncorhynchus kisutch*) via direct selection, found that sufficient genetic variability existed to improve the level of flesh colouration but cost limited application of this approach on a commercial level. Whatever the plan may be, a manager is presented with practical operational limits within which to conduct the project.

Unfortunately, $h^2$ estimates for the same trait can be highly variable among years and among stocks due to differences in environmental conditions (Bailey and Loudenslager 1986). Heritability values, calculated for other populations, are available in the literature (e.g., Gjerde and Gjedrem 1984; Tave 1986). These values provide some insight into whether a desired trait can be manipulated within a reasonable time frame to achieve the goals of the program. If heritabilities documented for the character of interest are low, it may take many generations to achieve desired results.

Brood programs strive to improve the phenotypic mean of the character of interest in the next generation. Documented heritability values can be used to calculate the amount of improvement to be expected. With this in mind, a program manager can establish limits for brood characters of interest. For example, assume that brood fish are identifiable by family (i.e., either individually marked or batch marked by family). Thus, measures of mean, variance, SD, and CV for specific traits of the parent stock should be attainable. Assuming that the operation will strive to meet minimum recommended numbers of brood fish, the program will be based on at least 100 fish of either sex. Matings of 100 pairs will result in 100 families. With a range of fecundities that reach several thousand eggs per female in some species of salmonids (e.g., *see* Chapters 2 and

3), it becomes obvious that it will be necessary to cull many individuals from these families before they become sexually mature.

For most breeding programs, it will not be feasible to maintain lineages of 100 families. However, it should be possible to maintain several of the best families of several spawners per family. The process of eliminating non-performers from the future brood stock is not limited only to the point at which the fish are about to be spawned. There are in fact several points at which stocks will have to be thinned. However, the important consideration is that poor performance at one stage does not necessarily mean the fish should be destroyed. Compensatory performance has been documented among salmonids that have had poor previous performance (Skilbrei 1990). An approach similar to that of Withler et al. (1987), in which the brood line is started with 1,000 alevins from each family, represents a workable compromise to accommodate hatchery space problems. These families should be ponded in separate tanks at least until they are large enough to be marked for subsequent recognition of family origin. After marking, it will be possible to trace the lineage of individual fish. This approach means that a final decision about an individual's desirability as a component of a brood stock can be delayed until maturation becomes imminent.

On the basis of measurements of desired traits both within and between families (or stocks when collecting wild brood fish), it will be relatively simple to identify both superior and inferior families as well as superior and inferior individuals within families. This concept is illustrated in Figure 3; Stock 1 appears to offer the best potential as it has the largest mean weight and a relatively high CV. Of course, obviously deformed individuals will be excluded from the brood program.

## MARKING BROOD FISH

Most breeding programs will require that brood stock be marked, either with unique or batch marks to facilitate breeding of non-related individuals of the next generation. Typically, the progeny of a particular mating will require a batch mark so that lineage can be identified at the time these offspring are ready to reproduce. It is not unusual for the best performers of a brood stock to be from a few families. A simple mass-selection, "superstimulus" hatchery mating approach risks a high frequency of brother-sister matings unless parental origin can be determined. In contrast, if a highly inbred strain is required, tagging is a means to assure that matings can be kept within a family.

A bewildering array of tagging apparatus is available for particular goals. McFarlane et al. (1990) presented a tabulation of multiple tagging techniques and stated that there is no single tag that is suitable for all organisms. Similarly, no single marking approach is appropriate to all applications for any given species. Arnold (1966) provided

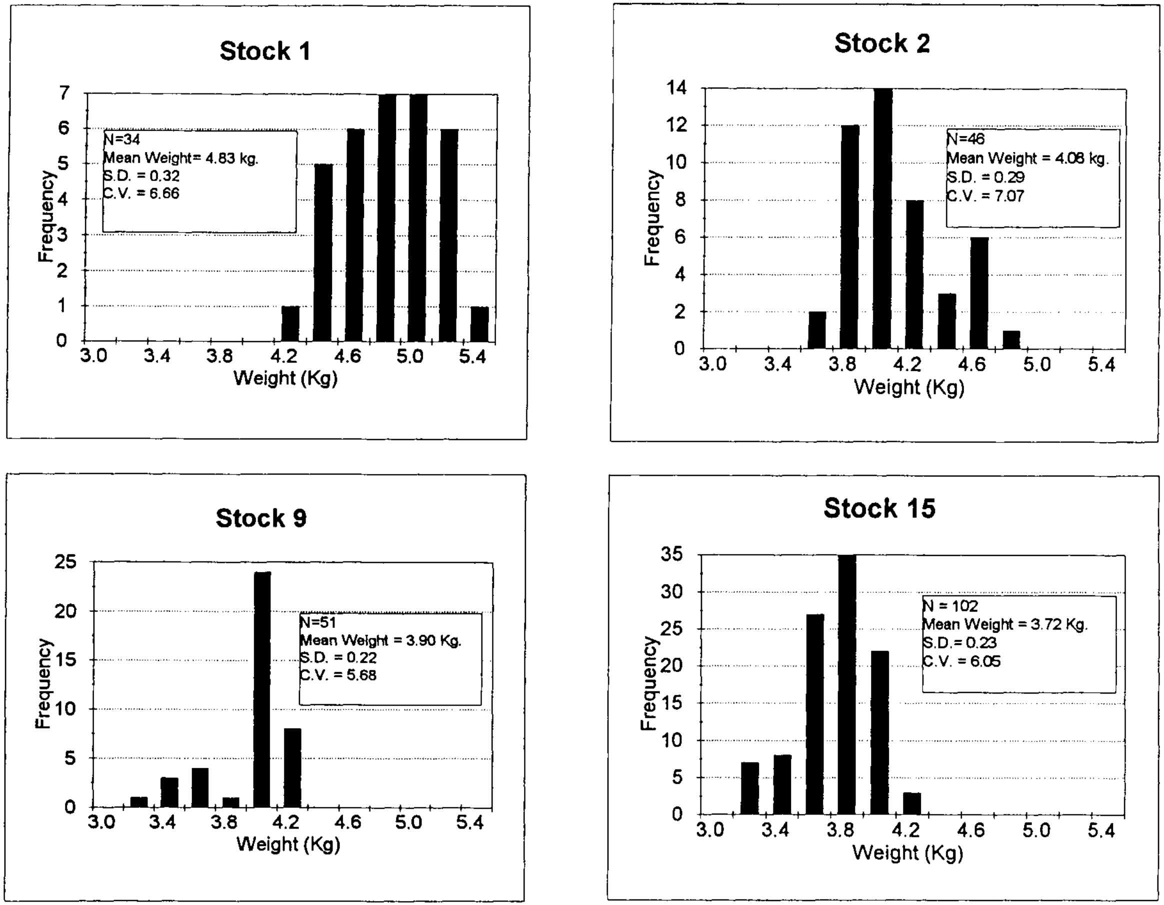

FIGURE 3. Population statistics from quantitative measurements provide useful tools with which to determine desirability of a stock for further brood development.

12 characteristics of an ideal fish mark but indicated that no marking device or agent known satisfies more than a few of these criteria. Several choices regarding marking techniques exist that require strict attention to the objective of the marking program and how important individual or family recognition is to the program. Of particular importance to broodstock management is the length of time that a fish must retain the tag or mark. Some marks range in longevity from days to decades depending on the species and the conditions of the study. It should be understood that tagging operations usually involve considerable trauma, both from handling of the fish and the wound caused by application of the tag (Morgan and Roberts 1976; Hall et al. 1984). Some mortality is likely and a manager must consider options carefully relative to potential benefits and costs.

An overall review of tagging techniques and their applications is beyond the scope of this chapter. For a general overview of marking techniques, the reader is referred to Parker et al. (1990). In the context of broodstock management, it is sufficient to emphasize five of the 12 criteria of Arnold (1966) as follows: (1) the mark should have no long-term adverse effect on the fish's behaviour, reproduction, life span, growth, feeding, movement, or vulnerability to predation; (2) the mark should not tangle in vegetation or nets of any kind; (3) the mark should be detected easily in the field by untrained personnel; (4) there should be enough possible variations of the mark so that many individuals or many small groups can be identified separately; and (5) the mark should not present any health or safety hazard, either to personnel, the fish, or the public.

After choosing a particular type of tag that will suit the broodstock program, a manager must consider how many fish should be marked and at what stage. It may not be necessary to mark all of the progeny from a particular mating. Only those progeny that will be retained for further consideration as future brood fish need to be identifiable. For anadromous species that have an extended freshwater rearing phase, tagging can be delayed until the last few months before parr-smolt transformation (smoltification) if sufficient hatchery space is available to maintain individual family lots.

## COLLECTING WILD BROOD STOCK

An essential component of the planning process that must take place before endeavouring to procure fish from a wild population is to establish the legal right to do so. In different parts of the world, access to salmonid populations may be privately controlled, such as with leased or owned waters, or public and controlled by government. Either way, written authorization should be procured before collecting fish from the wild. Typically, this authorization will include the terms and conditions under which the collection can take place.

There are at least three conditions for which wild brood stock may be required: (1) no commercial supply of eggs is available or the supply does not meet the needs of the program being contemplated; (2) legislation will not allow introductions of fish from outside the jurisdiction; or, (3) previous poor breeding practices have reduced the performance of the brood line and it is desirable to introduce the much greater genetic variability characteristic of self-sustaining wild stocks.

From baseline information, and calculations as per Tave (1986, Appendix B), one can determine how to select the required number of fish from the available population sample. This will reveal which specimens meet the required range for character performance and should be included in the program's brood stock, and those that should be returned to the wild stock. After deciding which salmonid stocks offer sufficient broodstock potential for the program, effective plans for brood stock capture will be required based upon a knowledge of the stock's life-history, behaviour, and the physiological stresses imposed on wild fish during their maturation cycle.

### *Interception of Wild Salmonids From Spawning Migrations*

Most salmonids return from their adult habitat to spawn in their natal waters. This presents opportunities to intercept potential brood fish both in river mouths and after they have entered the riverine environment. In most estuaries where there is a transition zone from salt water to fresh water, anadromous salmonids often will spend several days acclimating to the freshwater environment before actually proceeding upstream. At such transition times, and for several days after entry into the river, salmonids are under sufficient physiological stress (Maetz 1974; Pickering 1981*a*) that their capture is likely to result in mortality. Therefore, interception of potential brood fish in the marine environment is not recommended unless there is a significant and stable freshwater lens throughout much of the estuary. Baglinière et al. (1990) indicated that when adult Atlantic salmon reach the river, migration usually occurs in three successive phases: (1) rapid movement upstream for either long or short distances; (2) a long residence period; followed by, (3) a short upstream migration just before spawning. Spawning migration occurs with an increase in water flow (Banks 1969; Hellawell et al. 1974; Sandercock 1991) and all fish of a given species spawn at comparable sites from year to year (e.g., tail of a pool with large substrate size). To a large extent, spawning behaviour and the spawning habitat preferred by the species in question will determine what opportunities there may be to remove salmonids from their spawning stock.

Many salmonid hatcheries that rely on natural spawning runs for their brood fish have fixed structures to divert the fish from their upstream migration into traps and holding pools. Less costly methods for brood stock procurement, at least for short-term operations that are not repeated on an annual basis, include: angling (Senn et al. 1984); the mesh fence (Blair 1957); the fyke net (Tait et al. 1962); temporary counting fences (Kerswill 1971; Anderson and McDonald 1978); and diversion structures (Slaney 1977).

Brood collection from remote sites usually entails portable gear that, relative to the passive operation of weirs and dams (Hubert 1983), requires a greater degree of activity on the part of the brood collection crew. Capture of adult fish with sport-fishing gear sometimes is used for steelhead (*Oncorhynchus mykiss*) and Atlantic salmon. While angling can be a relatively cost-effective approach when pursued by volunteer groups, it usually is not practical where large numbers of eggs are required.

With chinook salmon (*Oncorhynchus tshawytscha*), which often spawn in deep riverine areas, it is common practice to use riverine drift nets to take fish that are in the process of spawning. These salmon are killed immediately and their gametes removed for transfer to a hatchery where fertilization is done under controlled conditions. Logistically, this approach has the advantage that brood procurement and egg take is confined to a relatively short time interval. However, not all salmonid species present this sort of opportunity. Many salmonids spawn in shallow stream habitat that is conducive only to hand seining. Complicated by rocks and stream debris, this can be difficult and greatly elevate egg mortality in redds that were constructed previous to seining. Accordingly, salmonids are often captured before the spawning season and held in artificial enclosures during the final maturation process.

Unfortunately, many salmonid spawning concentrations that will be of interest to a broodstock manager will be on river systems that are remote from hatchery facilities at which incubation will take place. This necessitates careful consideration of the disease spectrum of the potential donor population and of the recipient watershed or hatchery. Disease management is discussed in greater detail in Chapter 13. Hatchery managers do not usually risk contamination of their hatchery facilities by importing any fish or gametes of questionable disease status. Disease analyses of brood fish should be an integral component of a hatchery operation that must rely on eggs from any source for which the disease history is not known. Disease analyses of individual brood fish should be undertaken once the fish have been stripped of their gametes. Even if no diseases are found in the baseline survey(s), managers often will opt for holding and stripping salmonids at a remote location within the watershed in which wild brood fish are captured. Having once obtained the required number of brood fish from a wild population, normal inclination is to treat these fish for diseases that are of concern. This is not advised for reasons discussed later in this chapter. Quarantine incubation and early rearing facilities are advisable for operations that require collection of eggs from an uncertified source. Quarantine incubation should be conducted such that eggs from individual crosses are incubated separately, with no flow of water from one lot of eggs to any other lot.

The manager of a brood collection crew is well advised to avoid specimens that appear inferior in any way. This includes fish that may be lethargic or docile, are oddly coloured, or have evidence of mutilations. Fish that have open sores are not necessarily diseased. Such sores are often the result of abrasions from predators, parasites (e.g., sea lamprey, *Petromyzon marinus*), gillnets or jiggers and may heal if the fish is left in a pristine environment. Unfortunately, abrasions offer disease organisms an opportunity for systemic invasion and therefore provide a medium whereby diseases can proliferate and infect other fish. The confined conditions of artificial enclosures likely are more stressful to fish than are their natural environments. Higher densities of fish in artificial enclosures than are encountered in the wild provide ample opportunity for disease transmission.

There is a strong link between stress and disease susceptibility (Snieszko 1974; Barton et al. 1986; Maule et al. 1989; Pickering 1989; Barton and Iwama 1991). This underscores the need for optimizing conditions under which the fish are held to maturity. Security practices during brood maturation also are important. Acceptable conditions for holding salmonid brood stock through maturation will vary among locations according to the local adaptations of a given stock to its environment.

Conditions under which brood stock are being maintained in confinement are an important consideration. The broodstock manager must avoid conditions that may impede the maturation process. A wide range of solutions has been devised for temporary holding and for providing security for fish held in confinement (Klontz 1987, 1991; Moring 1989) including: (1) mesh baskets; (2) plastic pipes in which individual salmonids are placed and then submerged in the river; (3) tethering; and, (4) holding in cages of various design (Senn et al. 1984).

## REARING OF BROOD STOCK TO MATURITY

Reproduction is the most sensitive of all the life functions to stress (Gerking 1980). This is especially pronounced for females. Öogenesis, which begins as early as 12 months before spawning (Hunt et al. 1982; Sumpter et al. 1984; Youngson et al. 1988; McLay et al. 1992), may be the most sensitive stage in the reproductive process. Any stimulus increasing the drain on body energy resources (e.g., stress response, disease impacts), does so at the expense of gonad development. This attests to the importance of early planning of the broodstock rearing strategy. Ingram (1985) concluded that valuable brood stock must be carefully managed and treated delicately, even before the immediate pre-spawning period, and that stress should be avoided at all times. Much of the variability in egg quality documented in the literature may be attributable to fish culture practices. It is clear that good culture practices increase the potential for good gametes. Of significance is the observation that stress hormones (i.e., corticosteroids and catecholamines) appear to be normal components of the natural reproductive endocrine pattern in fish (Billard et al. 1981; Goetz 1983) and seem to play a natural role in the process of ovulation. This suggests that hormonal fluctuations due to the stress of excessive handling or aggression may interfere with reproduction.

As documented by Stanley (1989), the process of sexual maturation depends on three variables: time, water temperature, and photoperiod. Donaldson and Hunter (1983) indicated that culmination of the normal reproductive cycle may fail when environmental conditions (e.g., salinity, temperature, photoperiod, hydrostatic pressure, or lack of spawning substrate) are dissimilar from those prevailing in the natural environment during spawning. Gonadal growth requires time and the physiological processes that stimulate gonad development usually begin well ahead of any external manifestation of sexual characteristics. Progressive changes in photoperiod and temperature elicit the hormonal changes that stimulate gonadal maturation. Maturation requires that nutrients from body reserves be accessed and deposited in the gonads.

Besides the fact that they ought to be handled carefully, brood fish should be handled no more than is absolutely necessary. Handling causes physiological stress and all stress drains the finite energy reserve that a salmonid has during final maturation. In a situation of continuous stress, such as that from confinement, Campton et al. (1991) recorded that plasma cortisol rose rapidly to a plateau and remained there rather than increasing indefinitely. Billard et al. (1981) noted that potential brood fish are under both the stress of confinement and the normal physiological demands of maturation. Although individual stress factors usually are not lethal, multiple stresses are cumulative (Barton et al. 1986; Sigismondi and Weber 1988; Barton and Iwama 1991) and the resulting physiological load can result in mortality.

### *Optimizing Rearing Conditions*

The primary objective of salmonid broodstock programs is production of the maximum number of high quality eggs and fry from available brood stock (Bromage and Cumaranatunga 1988). It is notable that both egg number (fecundity) and the quality of eggs is related not only to the strain of brood stock but also to broodstock rearing conditions. These include husbandry and diet. Because fecundity becomes established early in the reproductive cycle, and as egg yolk proteins constitute > 80% of the egg dry weight (Wallace et al. 1987), food restriction at the time of development of the ovaries (vitellogenesis) may reduce egg production (Saunders et al. 1983; Thorpe et al. 1990). Besides the amount of food consumed, egg quality also is correlated to the quality of the diet. It is the quality of the diet that determines access of developing eggs to lipids, minerals and vitamins (Keong and Hung 1994).

The importance of appropriate rearing temperatures for maturation of brood stock is indicated from the studies of Korsgaard et al. (1986) who showed that the hepatic vitellogenic responses of post-smolt Atlantic salmon are strongly influenced by ambient temperatures. Another sign of suboptimal rearing conditions is an increase in egg atresia. Temperature anomalies may be responsible for a reduction in egg numbers at any time throughout the maturation cycle. Literature on the quality of eggs from first-time spawners indicates varying experience, ranging from egg quality that was better for virgin spawners than for repeat spawners (Hendrix 1989) to lower quality for first spawn eggs (Stevenson 1987; Shepherd and Bromage 1988).

Among wild Atlantic salmon populations in Newfoundland, there appears to be a greater incidence of repeat-spawning individuals that have inferior quality eggs relative to first-time spawners (V. Pepper, unpubl. data). The majority of these repeat spawners produce eggs that are of equal quality to virgin grilse. The reasons for this variability are uncertain but likely are related to fish health, nutritional state of the fish, and conditions under which brood are held during final maturation.

### *Water Quality and Stocking Densities*

The number of fish that can be carried in a given water supply depends on the water pathogen load, dissolved oxygen level, metabolic rate of the fish, feeding rate, and how fast the water is being exchanged. In turn, water exchange governs the rate at which metabolic wastes accumulate (Fox 1985). Actual working density at a given site will depend on temperature regime, water chemistry, and flow conditions. Generally, the higher the temperature (i.e., both average and extreme) and the lower the flow, the lower the workable adult holding density. For salmonid enhancement situations, wild brood fish usually are captured in a river system, likely well after they have ceased feeding. When retained in lotic (i.e., flowing) water conditions, brood stock can be maintained at relatively high densities. Senn et al. (1974) recommended the following holding densities ($kg/m^3$) for Pacific salmonids: chinook, 30-35; coho, 32; steelhead, 51; sockeye (*Oncorhynchus nerka*), 24. Pepper (1984) recommended 30 $kg/m^3$ as a holding density for Atlantic salmon brood under lotic water conditions and suggested 2.5 $l\ min^{-1}\ kg^{-1}$ of salmon as a satisfactory flow rate. For aquaculture brood while they are still feeding, more conservative holding densities are recommended. Ingram (1988) indicated that stocking density for brood stock should be lower than for production fish and suggested 8-10 $kg/m^3$ for rainbow trout as a maximum during the non-spawning season.

With uncontrolled exchange of water in net pens, there is the potential for introduction of pathogens, pollution, and noxious algae. Salmonids have natural mechanisms to help them cope with pathogens (*see* below) but avoidance of chronic pollution is one of the important goals of the initial siting (Landless and Edwards 1980). Unfortunately, blooms of noxious algae are more difficult to circumvent.

Phytoplankton blooms result from temporary unchecked growth of several species of unicellular organisms. These include dinoflagellates, diatoms, silicoflagellates, chloromonads, haptophyceans, coccolithophores and ciliates (Bruno et al. 1989). Blooms occur in response to a combination of climatic and hydrographic events and to the availability of nutrients, trace metals and vitamins. One of the prerequisites for blooms in Scottish waters has been a period of bright, warm, calm weather following a period of disturbed wet conditions.

Blooms of several species of dinoflagellates, some chloromonads and haptophyceans are known to produce toxins that kill fish and other marine organisms. Others such as *Chaetoceros concavicornis* and *C. convolutus*, normal components of the phytoplankton assemblage along the west coast of Canada (Albright et al. 1992), and *C. socialis* and *C. debilis* reported from Newfoundland (McKenzie and Deibel 1991), are particularly lethal. The spined setae of these species allow the cells to attach to the gill lamellae of confined salmonids where the barbs imbed in the gill tissue and break off (Martin and Wildish 1990). Despite the death of many of these diatoms, the silicified cells remain attached to the gill filaments. Severe irritation of the gills results in capillary haemorrhage, excess mucus production, necrosis, extensive sloughing of the gill lamellae and in consequence, respiratory failure (Bruno et al. 1989).

Most salmonid mortality attributable to oxygen depletion by algal blooms takes place during the night and early morning (Bromage and Shepherd 1988). At these times affected fish will be observed near the surface, gasping and exhibiting irregular gill movements. Depending on the offending species of phytoplankton, respiratory distress may be due either to physical damage to the gills or to high oxygen demand. Vast numbers of cells occurring in phytoplankton blooms will impose oxygen deficiencies at night or when there is insufficient sunlight to support photosynthesis. As well, when these blooms subside, decay of the enormous amount of phytoplankton material may severely depress oxygen levels for caged fish. It is noteworthy that algal toxins are among the most potent natural poisons in the world (Martin and Wildish 1990). Fish suffering from a toxic algal bloom show nervous signs and mortality that peaks during the hours of bright sunlight. Marine fish farms in the vicinity of massive dinoflagellate blooms stand to loose the entire crop. Losses attributable to such blooms have ranged from none to 100%. It appears that harmful algal blooms are becoming more frequent throughout the world. Alternatively, increased frequency may be an artifact of increased awareness due to expanding aquaculture and corresponding impacts of algal blooms on captive organisms.

In light of the above, and precautions pertaining to stress, it is pertinent to note the recommendations of Fox (1985):

(1) Maintain water quality within recommended guidelines for the species of interest;
(2) Regulate stocking density to prevent crowding stress;
(3) Recognize that multiple stress from crowding, handling, and disease treatments is additive or synergistic;
(4) When multiple stress is unavoidable, allow an adequate recovery time based on the species and severity of the resulting physiological disturbances; and
(5) Use the occurrence of stress-mediated fish diseases as an indicator of adverse conditions and take corrective action.

### *Temperature Regime*

Photoperiod and temperature are primary factors eliciting hormonal changes that stimulate gonadal maturation in temperate species (Lam 1983). While maturation is in progress, it is clearly a good idea to avoid extremes of environmental conditions. From the work of Saunders et al. (1983) and Thorpe et al. (1990) on Atlantic salmon has come the practice of combating "grilsification" by restricted feeding during the first winter in marine pens. Bromage et al. (1992) reported that rainbow trout maintained on low rations (0.4% of body weight per day) early in the maturation cycle exhibited 30-35% reductions in the number of maturing fish. Following periods of reduced feeding, up to 22% of the total number of vitellogenic oocytes may become atretic (Springate et al. 1985). Restricted food intake is likely to be an artifact of very low winter sea temperatures.

It is apparent that nutrition plays a key factor in gamete quality (*see* below). Fish that are expected to produce quality gametes must have access to essential nutrients throughout the maturation process, especially during the early maturation interval. Potential brood fish must deposit the nutritional precursors of gamete production in a physiologically accessible form. Such a reserve must be achieved well enough in advance of spawning to assure adequate body reserves before appetite diminishes. This requires active feeding that in turn requires adequate temperature. Physiological reserves of non-feeding fish are contained in the fatty deposits associated with pyloric caeca. It is important to understand that temperature limits for spawning and egg development are much narrower than the normal thermal limits for older fish (Elliot 1981). Gerking (1980) indicated that year-class strength may be determined in part by the temperature experience of the female during egg maturation, as öogenesis is the most sensitive stage of reproduction. Unfortunately, it is difficult to generalize about temperature recommendations between species or even among different stocks of the same species. For example, Isaksson (1991) pointed out that Atlantic salmon in Canada have an upper temperature tolerance several degrees higher than those in Iceland.

The best thermal strategy is simply a matter of avoiding extremes. While low sea temperature in the winter preceding spawning will deter the maturation process of Atlantic salmon confined to cages, extremely high sea temperature in the summer will interfere with physiological continuity of the maturation sequence. Extremes will cause stress. Reproduction is in itself a form of stress (Billard et al. 1981) and such stresses are additive. At best, environmental extremes will deter maturation, result in poor gamete quality and an extended spawning period in which there is greater opportunity for asynchrony between males and females. At worst, environmental extremes can cause mortality. Typical salmonid response to environmental stress is a sudden change in behaviour. As reported by Elliott (1981), there are three stages in the progression of abnormal behaviour as follows: (1) reluctance to feed, sudden bursts of activity with frequent collisions with the side of the enclosure, rolling and pitching, defecation and rapid ventilatory movements; (2) the fish become quiescent with short bursts of weak swimming, often floating on their sides or backs, may rapidly change colour and increase their ventilatory movements; and, (3) movements of opercula, fins and eyes are restricted and cease with death.

### *Feeding*

Wild anadromous salmonids discontinue feeding before entering fresh water. Their body reserves that can be mobilized in support of gonad development are fixed before they leave the marine environment. In contrast to the situation with wild brood, reproductive performance of a captive brood stock is dependent on the husbandry and nutritional practices of the operation. Diet influences egg quality by altering the chemical composition of the material deposited in the yolk (Hardy 1981) and therefore requires special consideration of ingredients to enhance egg quality. Nutrition influences reproduction by affecting fecundity, egg size and chemical composition, egg hatchability and fry viability, as well as survival of the brood stock (Eskelinen 1989).

Dependent on temperature and fish size, fish neither gain nor lose weight when fed at about 0.1% of body weight, a diet level defined as the maintenance ration (Purdom 1993). A fish that is not feeding (i.e., not achieving its maintenance ration) is deteriorating. Broodstock diet requirements are different from production fish. Brood stock usually are fed at 0.5% (Ingram 1985; Logan and Johnston 1992) to 1.0% (Bagenal 1969; Springate et al. 1985; Jones and Bromage 1987) of average body weight per day. This is a lower feeding level than is required for somatic growth of production fish. There appears to be no advantage of feeding potential brood fish in excess of 1.0% of body weight per day (Bromage et al. 1992). Of course, achieving this ration necessitates knowledge of the broodstock inventory (i.e., number and mean weight). It is rare that inventories of production fish at salmonid farms are accurate. Klontz (1991) documented an error of ±15-25%. Moring (1989) found an 8-38% error for individual locations with three to four cages each and an error of 2.5-46.5% for individual cages. There is a definite cause-and-effect relation between the nutritional status of the brood stock and the quality of the reproductive products, thereby implying that additional effort to assure accurate inventory of broodstock animals is warranted.

For a brood stock being fed at only 0.5% of body weight per day, it is likely that much of the ration will be consumed in very few feeding periods. For salmonids in general, both appetite and swimming speed have the same daily rhythm, being highest in early morning and evening, and lowest in early afternoon (Kadri et al. 1991). The disposition of the brood stock towards feeding at any one time will determine whether the daily ration should be applied in a single feeding or not.

As feed is the greatest ongoing cost factor in operating a salmonid farm, it is important to emphasize efficiency of feeding. Ritter (1989) stated that large deposits of adipose fat can interfere with the spawning process. This observation suggests that quality of the broodstock diet is more important than quantity. Present feed strategy on many commercial farms is to mix diets from different manufacturers. This, coupled with methods of quality control practiced by most feed companies, makes it unlikely that diet deficiencies in present day aquaculture would contribute to gamete inferiority. Relative to grower diets, broodstock diets usually contain higher levels of trace elements (zinc and manganese), higher concentrations of both water- and fat-soluble vitamins, and lower levels of dietary protein and lipid (Ritter 1989). Much progress has been made in formulating broodstock diets by examining whole-body, essential amino acids (EAA). Essential amino acid profiles of maturing fish have been examined to determine the ideal EAA requirement pattern (Wilson and Halver 1986). However, EAA studies of larval fish are needed to determine if the EAA of eggs can be used as formulation guidelines for broodstock diets (Keong and Hung 1994).

Present strategy for most fall-spawning salmonids is to build up body energy reserves in the spring and early summer. Bromage et al. (1992) reported that groups of rainbow trout fed high rations for the first four months of the maturation cycle had the highest percentage of spawned fish and some of the highest fecundities. Relative fecundity significantly increased as a result of the lower rates of growth in the three months leading up to spawning. Ordinarily, feeding of brood fish is stopped several weeks in advance of spawning, thereby shutting down the digestive process and allowing the digestive tract to be thoroughly purged. This also reduces feeding costs.

Digestion in salmonids is primarily enzymatic rather than microbial (Klontz 1987). Hence, salmonids do not rely on intestinal microflora to extract the biochemical energy contained within their food but rather, they must have all the digestive enzymes required to break down food ingredients into the simpler molecules required for salmonid metabolism (*see* Chapter 10). This partly explains why fish do not make efficient use of carbohydrates and why some protein is metabolized for energy (Lall 1991). Effective diet formulations are those that recognize the salmonid enzyme spectrum to the end that feeds are formulated so as to be digestible by these enzymes. Considerable heat can be generated in the salmonid digestion process and continuous passage of food materials through the gut is important. If passage of food materials through the digestive tract is interrupted, heat can damage the mucous membranes lining the digestive tract (Klontz 1987). Reduced intestinal mobility is one effect of physical handling of the fish and this can result, not only in sloughing of the mucosal lining, but in small intestinal haemorrhages. In turn, these haemorrhages provide systemic access for bacteria that are normal components of the gut. Intervals of about two or three days in which food is withheld from fish that must be handled should minimize the likelihood of static gut problems.

Studies on broodstock nutrition have revealed the role of vitamins, trace elements and essential fatty acids in reproduction (Lall 1991), to the end that the risk of prespawning mortality can be greatly reduced by special broodstock diets. Watanabe et al. (1984) showed that a low protein diet has no adverse effect on reproduction if sufficient energy is supplied by the lipid supplement. Protein requirements decrease from about 50% for salmonid fry and parr to about 30 to 40% of the diet at the later stages of development (Lall 1991; Watanabe et al. 1984). With a diet containing 21% lipid, this reduced level of protein was sufficient to produce quality eggs with good hatchability (Watanabe et al. 1984).

The role of trace elements (manganese, iron, zinc and copper) and vitamins (ascorbic acid, vitamin E) also has been demonstrated in reproduction (Watanabe 1985). Shelf life of brood diets is important. Broodstock feed should contain at least 100 mg/kg ascorbic acid at the time of feeding (Eskelinen 1989) and a dietary vitamin E level of 3.4 mg/100 g (Takeuchi et al. 1981) if levels comparable to those observed in wild spawners are to be reached. Dietary vitamin E has been observed to be efficiently transferred to eggs and fry. As with vitamin E, fatty acid distribution in eggs is readily affected by dietary lipids. Watanabe (1985) demonstrated that addition of linolenic acid to broodstock diet deficient in essential fatty acids (EFA) led to marked improvement in egg fertilization, percentage of eyed eggs and total hatch, compared with brood stock given diets lacking EFA. Leray et al. (1985) reported that during öogenesis, dietary lipids appear to be diverted from adipose tissue and deposited in the eggs. Adequate levels of EFA are critical to normal spawning and to ensure good hatchability of eggs. Various pigment substances (carotenoids) also may be important components of broodstock diets though the relationship between carotenoid content and quality of broodstock progeny is not so far resolved (Torrissen 1984; Tveranger 1986). Active metabolism of carotenoid pigments suggests these have an as yet unquantified function in reproduction.

For some salmonid programs, particularly aquaculture operations, brood stock will be available either as a result of controlled selection of fish that have been spared from marketing, or, for some salmonids, as a result of brood reconditioning from previous years. Kelt (i.e., previously spawned salmonids) reconditioning techniques have been developed for Atlantic salmon (Gray et al. 1987; Pepper and Parsons 1987; Dumas et al. 1991; Crim et al. 1992; Johnston et al. 1992). Atlantic salmon brood fish in fresh water may produce eggs many times during their life. For example, an Atlantic salmon brood stock at the Cardigan Fish Hatchery, Prince Edward Island, Canada has produced quality gametes in 14 successive years (Randy Angus, Cardigan Hatchery, P.E.I., pers. comm.). For most commercial operations however, kelt reconditioning is considered unwarranted, too costly, or logistically impractical.

## DISEASE (*see also* Chapter 13)

Loss of valuable broodstock to disease is of course a serious risk to any broodstock program. This risk is increased as maturing fish tend to be easily stressed and have somewhat compromised immune systems. A related risk is the vertical transmission of some diseases to the progeny usually through the incorporation of the pathogen into the egg during final maturation. Diseases can also be transmitted to progeny on the surfaces of eggs if eggs are not disinfected (*see* Chapter 5). Most of the diseases of concern to broodstock managers are discussed in Chapter 13, but of special concern are several pathogens and parasites which attack the epidermis of maturing fish and these are discussed in detail here.

### *Fungus and Other Skin Conditions*

In salmonids, saprolegniaceous fungal infections often are associated with sexual maturity (Roberts and Shepherd 1974; Wood 1974; Neish 1977), there being a greater incidence of infection in sexually mature males than in females before spawning (Ingram 1985). Such infections can be serious. Richards and Pickering (1978) reported that *Saprolegnia*-infected brown trout from a wild population had 20-25% of the body surface covered with fungus at the time of capture and up to 50% of the body surface of hatchery-reared brown trout was infected. Differences in degree of infection with gender may be due partly to the behavioural tendency of males to fight in the presence of females (White 1975) thereby resulting in wounds to which fungal spores become attached. Neish (1977) considered that *Saprolegnia* sp. infecting sockeye salmon are pathogens capable of invading living tissue and, in some cases, the fungus may be the primary infectious agent causing lesions. It appears that once fungus is established, it first destroys the epidermis and, as the infection progresses, fungal hyphae subsequently penetrate into the musculature. As salmon and trout are exposed to potentially parasitic saprolegniaceous fungi throughout their stay in fresh water, Knittel (1966) concluded that fungi are facultative parasites. The ability of salmonids to cope with fungus infection is dependent on their nutritional state and stress. Again, maximizing the former and minimizing the latter are part of good management. For a confined stock, provision of a high quality diet throughout the early maturation stages is important. For wild salmonids, attaining sufficient physiological reserves prior to cessation of feeding during final maturation provides the nutritional basis of fungal resistance.

In a freshwater environment, fungus can be treated by a salt bath (Avault 1980) that, for extended immersion, should be at 10‰ as this salinity is nearly isotonic with salmonid body fluids (McCormick et al. 1989) and therefore should not impose undue osmoregulatory stress. For fish that are ready for transition to marine conditions, Gibson (1982) suggested 18‰ would be sufficiently high to prevent fungus attack, require little additional metabolic maintenance, and allow normal hyper-osmotic kidney and gill function. As a dip, salt concentration should be about that of seawater (i.e., ≈32‰; Piper et al. 1982). While there are several chemicals that are effective as fungicides, salt is a relatively environmentally benign substance. Malachite green has been an effective treatment (Knittel 1966) but now has fallen into disfavour due to environmental concerns and reputed carcinogenic properties. Murphy (1981) indicated that the chemicals methallibure and cyproterone acetate are effective means of combating fungal infection.

Prolonged exposure to solar ultraviolet (UV) radiation has resulted in significant losses on salmonid farms. As a result of epidermal damage due to the effects of sunburn, opportunistic pathogens, normal components of a cage environment, are presented with an ideal means of systemic invasion. The sunburn effect is the basis of the "summer syndrome" noted by McArdle and Bullock (1987). Anecdotal observations suggest natural populations avoid the sunburn problem by tending to enter rivers at times of increased flow when the rivers are most turbid. Sunburn in aquaculture can be avoided by provision of some form of shade. Ultraviolet penetration into water can be impaired also by spraying a water mist over fish confinement areas, thus creating surface agitation and scattering UV wavelengths.

Skin lesions in salmonids also can be caused by parasitic external copepods that in high numbers can impair physiological performance and increase susceptibility of captive fish to disease. Gall et al. (1972) recorded decreased fecundity due to a copepod infestation in rainbow trout. Sea lice, *Lepeoptheirus salmonis* and *Caligus elongatus*, constitute one of the most serious problems for pen-reared Atlantic salmon (Smith and Clark 1988; Kent 1992) and their presence on large numbers of farmed Atlantic salmon is well recognized as a serious cause of losses in marine fish farming (Wootten et al. 1982). Adverse environmental consequences of organophosphate baths used to combat sea lice recently have lead to the alternative use of effective oral preparations (Smith and Clarke 1988). Another alternative and novel approach to sea lice control is documented by Bjordal (1990) who suggested that two or three different wrasse (Labridae) species might be used as cleaner fish for effective parasite control in commercial salmonid farming. However, some species of wrasse are sufficiently aggressive to pick the eyes out of otherwise healthy aquaculture salmonids once their ectoparasite targets have all been consumed. The number and species of cleaner fish introduced into salmonid cages should be closely monitored and care also should be exercised to assure that such cleaner fish are not themselves infected with some sort of pathogen or parasite.

### *Prophylactic Treatment of Aquaculture Brood Stock*

Contrary to advice on wild broodstock, captive brood stocks should be subject to preventative treatment whenever there is threat of loss due to disease organisms. Brood fish are simply too valuable to the future of a salmonid farm to risk their loss. Implicit in this argument is the expectation that brood fish will achieve spawning condition as a result of good management and that one aspect of this management is site visits by a fish health specialist. The extra expense of ongoing security, special diets and disease treatments are wise investments when the future of the endeavour is dependent on these brood fish. A salmonid farm that has sustained the expense of maintaining fish throughout their lives, likely as separate family groups or lines that have been selected as the best performers, simply cannot afford to jeopardize the breeding strain by taking chances on environmental variables. The goal of a broodstock operation is production of quality offspring. This goal depends on obtaining quality eggs, which is dependent in turn on maintaining a healthy brood stock through the final stages of maturation. A brood stock that is suffering the stress of combating disease, even if the disease is not lethal, will have a reduced probability of producing quality offspring due to diversion of energy from reproductive development.

In the case of wild brood stock, the financial investment in collecting brood fish, though significant, is likely to be small relative to the cost of constructing and operating a hatchery. As wild brood stock represents an unknown risk of disease contamination for hatchery facilities, it is important to sample the brood fish to determine their disease spectrum. This is best accomplished by lethal sampling of the brood fish at the site of capture immediately after stripping eggs. By applying individual marks to brood fish at the time of capture, and incubating the eggs from each female as discrete and labelled units in isolation of those from other breeding pairs, it will be possible to identify the pathogen history of each family. Lethal sampling of all parents and separate incubation by family lot until the results of disease analyses are available (likely four to six weeks) potentially will allow elimination of families that represent unacceptable disease risks. Surface disinfection of fertilized and water-hardened eggs can be effective in eliminating external bacteria. However, if any crosses are found to have pathogens dangerous to the continued operation of the hatchery (i.e., either something that is not already present in the hatchery or water supply, or something that can be vertically transferred from parents to offspring in the eggs), individual lots can be destroyed without necessarily losing the entire egg take.

The rationale for recommending not to apply antibiotics or vaccine to newly captured wild brood stock is that such treatment may render subsequent disease analysis of these fish meaningless. Such disease treatments provide brood fish with the means to combat pathogens, but do not necessarily destroy the pathogens. However, such treatments are likely to interfere with laboratory analyses of the brood carcasses, thereby decreasing effectiveness of the disease screening. The basis for recommendations on treatment of brood fish of hatchery origin, vs. no treatment for those of wild origin, is both a matter of cost and of risk. The cost of maintaining a brood stock through its entire life cycle is too great to risk its loss due to disease. If a new brood line is found to be contaminated, therefore presenting an unacceptable risk to the continued operation of the hatchery, the approach is to destroy the new line, disinfect the quarantine facility, and accept the cost of purchasing disease-free eggs from another hatchery. Such Draconian measures should be viewed as an interim measure to sustain the operation until another wild population can be accessed.

Disease concerns and combative strategies will be well served by hatchery quarantine facilities in which eggs from locations external to the hatchery can be maintained in isolation from other hatchery facilities. A logical premise for a quarantine facility is "no pathogens in, and no pathogens out". Depending on the hatchery site and its sources of water, there may be suitable options for achieving this. No pathogens could imply: 1) a groundwater source with sterilization of the effluent; 2) a groundwater source with recirculation; 3) water sterilization of both incoming and discharge waters; or, 4) sterilization of incoming surface water in a recirculation operation. A quarantine facility should have sufficient capacity to support fry rearing for a period of at least four months after first feeding (i.e., until the fish reach about 5 g). This will provide the opportunity for further analysis for diseases that can be vertically transferred from the parent fish. No fish health analysis is absolutely certain (i.e., there typically is a 5% risk of not detecting a pathogen). If the parents are pathogen-free, and further analyses indicate resulting progeny are clean, the risk of undetected disease is greatly reduced. Local regulatory agencies, extension services, veterinarians, and fish health personnel should be able to advise on construction of quarantine facilities.

### *Pharmaceuticals*

Chemicals of potential application to aquaculture fall into four main categories: (1) feed ingredients; (2) biologics; (3) drugs; and, (4) pesticides. Though definitions vary somewhat among countries, the notion of what constitutes a drug requires some clarification. In Canada (*Food and Drugs Act*, R.S., c.F-27, s.1, 1989), drugs are considered to include "any substance or mixture of substances manufactured, sold or represented for use in: (a) the diagnosis, treatment, mitigation or prevention of a disease, disorder, abnormal physical state, or the symptoms thereof, in man or animal; (b) restoring, correcting or modifying organic functions in man or animal; or (c) disinfection in premises in which food is manufactured, prepared or kept." (Carey and Pritchard 1995). In the United States, further distinction is made between low and high regulatory priority drugs. Within the U.S. jurisdiction there are guidelines for application of such intuitively benign compounds as sodium chloride and sodium bicarbonate. Currently, there are 17 compounds identified within the U.S. as low regulatory priority. Under the U.S. Food and Drug Administration, there are only seven compounds listed as Approved New Animal Drugs (Rosalie A. Schnick, National Fisheries Research Center, La Crosse, WI, pers. comm.).

An aquaculturist should assume that any list of approved chemicals will change with time and therefore, should be reviewed on a regular basis. The regulatory climate in the U.S. concerning use of any compound for treating aquaculture animals, particularly those destined for human consumption, should be scrutinized by any fish culture operation expecting to access the U.S. market (Kramer 1986). Present U.S. regulations may soon apply to products imported into that market. Regardless of the regulatory jurisdiction under which a particular fish culture operation must function, it is wise for the aquaculturist to maintain a current understanding of restrictions that apply.

It is likely, at one time or another during the course of annual salmonid culture operations, that the fish culturist will have to rely on various chemicals to avoid excessive fish mortality. While some aspects of disease management have been discussed above as they pertain to brood stock, a more comprehensive discussion is presented in Chapter

13. In the specific context of brood stock, further consideration of pharmaceuticals relative to brood stock issues is limited to anaesthetics.

### *Anaesthetic Chemicals* (*see also* Chapter 12)

The use of anaesthetics is virtually an everyday occurrence where fish must be handled. Anaesthetics are desirable for at least three reasons: (1) reduce stress effects on the fish (Brooke et al. 1978; Strange and Schreck 1978; Kent 1992); (2) avoid internal damage to the fish from rough handling that may be required by attempting to manipulate an active fish (i.e., provide humane restraint); and, (3) suppress perception of noxious stimuli (Green 1979). All general anaesthetics act as central nervous system depressants (Bell 1967) and according to Siwicki (1984), the majority of anaesthetics are strongly toxic to fish. Soivio and Nikinmaa (1981) found that erythrocytes of rainbow and brown trout swelled when the fish were anaesthetized with MS-222 or benzocaine. Eddy and Morgan (1969) indicated that carbon dioxide can be toxic to fish and that toxicity is usually associated with its effect on fish haemoglobin. The toxic effect of $CO_2$ is due to reduced affinity of the blood for oxygen (Eddy and Morgan 1969). If $CO_2$ is to be used for anaesthesia, it should be accompanied by oxygen infusion into the water bath and pH buffering. Billard (1981) found that of three anaesthetics (i.e., phenoxyethanol, quinaldine, MS-222), only phenoxyethanol showed a toxic effect on fertilization. The effect was limited to the sperm and did not appear to affect eggs.

Anaesthetics themselves may be stressful to fish at certain dosages (Barton and Iwama 1991). This suggests moderation in their use, careful attention to concentration of anaesthetic solutions, and control of the duration of exposure. Of course, anything that is toxic to fish should not be used on fish that are destined for market in the near-term. As an example, anaesthesia with 2-phenoxyethanol results in its distribution in the liver, kidney, gall bladder, brain, and especially the cerebellum (Imamura-Kojima et al. 1987). Fortunately, the half-life of these residues is only 30 minutes.

Stoskopf (1993) indicated that anaesthesia provides immobilization, relaxation, unconsciousness and freedom from reflex response, as well as relief from pain. The following characteristics of the ideal anaesthetic agent for fish are taken from Stoskopf (1993):

(1) renders them insensible to pain;
(2) provides good immobilization and muscular relaxation;
(3) induces unconsciousness, tranquillization or sedation in a predictable manner related to dose;
(4) is easily administered;
(5) induces anaesthesia quickly;
(6) provides a rapid, predictable and uncomplicated recovery;

(7) has a wide margin of safety — the dose that will cause irreparable harm or the death of the fish should be much greater than the dose required to achieve suitable anaesthesia;
(8) is inexpensive — leaves no tissue residues and requires no withdrawal times before the anaesthetized fish can be brought to market; and
(9) uses chemicals that are stable, have a long shelf life and break down rapidly in the environment to safe and physiologically inactive metabolites.

As the use of various pharmaceuticals for livestock is subject to rapid change by governing regulations (e.g., *Food and Drugs Act*, Canada; *Federal Food, Drug, and Cosmetic Act*, United States), local veterinarians or fish health specialists should be consulted to provide current advice on use of anaesthetic chemicals for fish. At least 15 anaesthetic agents have been used in fish culture (Bell 1967; Piper et al. 1982; Bell 1987). Of these chemicals, quinaldine (2-methylquinoline), tricaine methane sulphonate (MS-222), and benzocaine are the most popular fish anaesthetics. Ross and Ross (1984) gave general precautions and considerations in the use of sedation-inducing chemicals in fish.

Anaesthetic chemicals can be used in a water bath or as an intraperitoneal injection. However, caution is advisable. Iwama et al. (1989) warned that anaesthetics administered under unfavourable conditions can cause stress. One example is unbuffered carbon dioxide gas that acidifies the water bath. When fish are placed in water baths containing anaesthetics, anaesthesia results when the fish absorb the sedation chemical through the gills and partly through the skin. With properly administered anaesthetics, stress is associated more with handling during capture than with the hypoxia that accompanies deep anaesthesia. While anaesthesia can itself be a very potent stressor, Iwama et al. (1989) found that the effect of netting and handling the fish was the cause of stress in their studies rather than exposure to the anaesthetic treatments.

With a few precautions, carbon dioxide can be a satisfactory anaesthetic for fish not requiring repeated anaesthesia. This is a particularly common practice with aquaculture operations at the time of harvest. In gaseous form, $CO_2$ should be used only in concert with a buffer (e.g., sodium bicarbonate) to prevent progressive acidification of the anaesthetic bath. The fish culturist should be aware also that the pH of water and weakly buffered aqueous solutions decreases with increasing temperature (Moyle and Cech 1988). Precautions to maintain a stable anaesthetic bath are advisable. Household baking soda ($NaHCO_3$, Booke et al. 1978) is a relatively safe means of $CO_2$ anaesthesia that presents little threat to human health. An effective $CO_2$ anaesthetic bath should be achieved with a $NaHCO_3$ concentration of 142 to 642 parts per million. For stripping of brood stock, alternative means of sedation may be more appropriate (Billard 1981; Gilderhaus 1990). Regardless of the means of anaesthesia, it is good practice to blot the brood fish with absorbent material (e.g., cloth or paper towel) to remove traces of the anaesthetic chemicals that may adhere to the body of the fish and otherwise may drip into the gamete container during stripping. Alternative anaesthetics referenced in the literature are presented in Table 2. Dosages are discussed in Chapter 12.

## FINAL BROODSTOCK MATURATION

The ultimate goal of every broodstock operation is production of good quality eggs and offspring. Maintenance of brood stock in good condition is a major concern in salmonid aquaculture because of potentially high prespawning mortality, large numbers of fish that do not mature when predicted, and poor incubation success from inferior gametes. Literature on incubation and early rearing survival confirms highly variable results. Briggs (1953) documented average hatchery survival to the eyed-egg stage of 77-78%. Springate and Bromage (1983) recorded fertilization and hatching rates of salmonid eggs in hatcheries of 90% and 70%, respectively, but subsequent survival up to four months of age of only 35-40%. Bromage and Cumaranatunga (1988) recorded highly variable results ranging from 0% fertilization to 85% survival to the four-month, fed-fry stage. Such observations suggest there is a multitude of factors (Billard 1992), both intrinsic (e.g., genetic, physiological) and extrinsic (e.g., diet, holding conditions) that should be optimized in an attempt to procure quality gametes that produce a high incidence of fertilized eggs with good survival potential.

As noted above, environmental conditions during the final stages of maturation are important and disruption of normal environmental cues may impact on brood performance. Brood fish are reported to mature later in the year and over a longer period in sea cages (Needham 1988). Sower and Schreck (1982) found that maturation of coho salmon brood stock in salt water, even when induced by hormones, met with little success. Allee (1981) added that saltwater maturation is not satisfactory relative to freshwater performance standards and that both males and females contribute to infertility in salt water.

Experience with Atlantic salmon in marine cages in Newfoundland has been variable among years (V. Pepper, unpubl. data). Given careful feeding with a quality diet, a significant and stable freshwater lens, a low-stress environment in which both males and females are able to interact throughout the maturation interval, and a brood strain only one generation removed from the wild stock, marine cages have been used effectively in Newfoundland (i.e., <5% failure rate) to accommodate Atlantic salmon brood throughout the maturation cycle. Egg quality, as judged by uniformity of egg size, colour, stage of ripeness, fertilization success compatible with any self-sustaining wild salmonid stock, and about 90% green egg to early parr survival, confirms that there is no absolute prohibition of brood-stock holding in marine cages, a conclusion also reached by Wertheimer (1981). In contrast, Atlantic salmon farms in the Bay of Fundy on Canada's east coast have experienced low gamete viability from brood stock retained in full salinity seawater. Under such conditions, industry expects only one third of the eggs

**TABLE 2**

Some anaesthetics for fish identified in published literature.

| Chemical | Reference |
|---|---|
| Benzocaine | Gilderhaus 1990 |
| Carbonic acid gas ($CO_2$) | Post 1979 |
| Propanidid-POLFA | Siwicki 1984 |
| Quinaldine (2-methylquinoline) | Stoskopf 1992 |
| Tertiary amyl alcohol | Bell 1987 |
| tricaine methane sulphonate (MS-222) | Marking 1967 |
| 2-phenoxyethanol | Klontz and Smith 1968 |
| Metomidate | Kreiberg 1992 |

collected in any one year to survive to the early parr stage (Gerry Friars, Salmon Genetics Research Centre, St. Andrews, Canada, pers. comm.).

Baynes et al. (1981) and Billard (1983) indicated that high reproductive fluid osmolarities adversely affect spermatozoa motility. A large amount of coelomic fluid is produced during ovulation in salmonids. As ovulated eggs lie free in the body cavity, and the ion concentrations and osmolarity of the coelomic fluid fluctuate in parallel with changes in the plasma values (Hirano et al. 1978), it is reasonable to assume that a changing external salinity regime will affect the internal environment of the eggs as the brood fish attempt to compensate. Rao (1981) noted that changing salinity increases the energy cost of osmoregulation in rainbow trout and is greater in full salinity seawater than in water that is isosmotic with the body fluids. Such increased energy costs divert valuable physiological energy away from gonad and gamete development.

The normal period over which spawning occurs for many species of salmonids is three to four weeks. Within this interval, one can expect considerable variation in the state of maturity among individuals. Although it is important to minimize handling and avoid disturbing fish during the prespawning interval, brood fish will have to be examined to determine when they should be stripped. Experience will determine when a salmonid stock can be expected to spawn. A random sample of 25 female brood fish should be examined once each week as the spawning season becomes imminent. Stripping should proceed about two days after 20% of the brood fish (i.e., 5 of the sample of 25) are ripe. This scrutiny is an unavoidable trade-off between imposing handling stress and the consequences of overripe eggs. The need for handling is greatly reduced when history of the spawning interval for the particular hatchery stock is known. Unfortunately, pronounced changes in environmental conditions (e.g., due to volcanic eruptions; El Niño; etc.) may profoundly affect spawning time, even in known brood stocks.

Timing of gamete collections from maturing brood stock is important. The only factor that has been shown clearly to affect egg quality in rainbow trout is the time of stripping of fish in relation to ovulation (Bromage et al. 1992). For example, the capacity of an egg to be fertilized depends upon completion of meiosis. This process begins before ovulation but the öocyte does not complete meiotic maturation until some time after fertilization (Nagahama 1983). Final öocyte maturation involves dissolution of the nuclear membrane (germinal vesicle breakdown) upon resumption of meiosis, and achievement of the second meiotic metaphase stage (Mylonas et al. 1992). Only at this stage is an öocyte first considered fertilizable.

From the practical side, Needham (1988) reported that appropriate stripping time for salmonids occurs in the four days following egg release from the ovary. Quality of the egg deteriorates with time after ovulation. Springate et al. (1984) reported that eggs stripped between four and ten days after ovulation at 10°C consistently achieved high rates of fertilization. After 20 days, only a few eggs were capable of being fertilized and of those, few survived incubation. In contrast, Fitzpatrick et al. (1987) found the highest mean viability of coho eggs 20 days after ovulation. Hynes et al. (1981) reported that ova may be kept up to 30 days *in vivo* for large rainbow trout females but only 15 days for younger females. However, Hynes et al. (1981) also indicated that maximum egg and fry survival are achieved if the ova are stripped four to six days after ovulation. Carpentier and Billard (1978) reported that survival time of eggs after *in vitro* preservation is three days at 0°C. Egg survival in dead females is variable, from as short as a few hours (Billard et al. 1980) to as much as 12 hours (Ingram 1985). Scott and Baynes (1980) reported that sperm taken from Pacific salmon between 1.5 to 5 hours after death were still completely viable. Those authors indicated that although spermatozoa initially are unable to activate when retained in this way, they recover on exposure to the air for 10-15 minutes. Ingram (1985) indicated that milt has been used with no loss of viability for up to 72 hours after death.

Salmonids have a variety of spawning times that vary with species (Chapter 3). Most (e.g., Arctic charr, *Salvelinus alpinus*; Atlantic salmon; brook trout, *Salvelinus fontinalis*; brown trout; chum, coho and pink salmon; and some strains of rainbow trout) tend towards fall spawning and undergo their final maturation under conditions of decreasing photoperiod and temperature. Alternatively, some (e.g., cutthroat trout and some strains of rainbow trout) spawn in the spring and undergo final maturation during conditions of increasing day length and water temperature. It is this predictable cycle for a given stock that allows the manager of a salmonid brood program either to work within the natural reproductive cycle or to manipulate the cycle to produce gametes when they are needed for the particular program. Either way, potential brood fish must be treated with care to provide adequate numbers of quality gametes. Preparations for spawning vary considerably among hatcheries according to the exact conditions under which brood fish are being held. Most salmonids have a natural repertoire of stimuli that synchronize maturation to a time of year that maximizes survival potential of offspring in their natural habitat (Brannon 1987). In addition to environmental stimuli noted above that synchronize spawning to a particular time of year, interactions among fish both within and between the sexes serve to synchronize spawning within the brood stock (Liley et al. 1986). Reproductive potential will be maximized when all of these stimuli act concurrently. If any of these natural stimuli are obstructed, such as by placing brood fish in artificial enclosures, maturation commonly is delayed or the spawning interval is increased. This can lead to variable gamete quality over the duration of the spawning interval.

Male fish aggression is a normal aspect of natural spawning behaviour. During maturation, male salmonids develop an enlarged and hooked lower jaw (kype) that serves as a weapon in competition with other males. Concurrently, they also develop a thickened and greatly toughened integument that serves to deter serious injury during competitive interactions for females on the spawning grounds. This is an extremely important example of genetic coadaptation (Falconer 1989) that conveys survival potential to the species. Under natural conditions, this genetic adaptation acts in concert with behavioural mechanisms to establish dominance hierarchies whereby subordinate fish can retreat from combat and live to compete another day. Under conditions of artificial confinement, where retreat from a loosing battle may not be possible, serious damage may result, both to the combatants and to the non-participants. Under some conditions of artificial confinement, when spawning is imminent, males and females may have to be separated to avoid stress from fighting males within the brood stock.

In situations where brood stock must be held in marine cages, a freshwater lens seems to be important. In the absence of a spawning substrate, it may be important also to have males and females together to stimulate the final stages of maturation. A manager of a broodstock program is well advised to observe the fish carefully to determine their behaviour and thereby interpret an appropriate strategy for final brood maturation and procuring gametes.

Pacific salmonids die after spawning. While Atlantic salmon and the trouts and charrs can spawn several times, most hatchery operations do not use brood fish more than once (Hynes et al. 1981). Under certain conditions, such as when a brood stock is retained at a remote location and weather conditions are unsatisfactory for stripping, it may be appropriate to kill ripe brood fish before gamete collection so that they can be removed to a more convenient location. As noted above, depending on their time of ovulation, *in vivo* eggs can remain viable for up to 12 hours after death (Ingram 1985). The appropriate precaution here is that corpses must be kept cool with crushed ice if available. Unfortunately, this approach allows no margin for error. Once killed, a brood fish that has not fully completed the maturation process could represent a significant financial loss to the brood program. Yet another precaution pertains to the method of euthanasia, which should be as humane as possible. An overdose of $CO_2$ is a relatively benign technique that, under controlled conditions as noted above, is not toxic either to eggs within the body cavity or to the environment. In contrast, any technique that causes a sudden and violent contraction of body musculature can rupture blood vessels and result in internal bleeding. Chapter 5 provides greater insight into the fertilization and incubation aspects of salmonid culture.

### *Synchrony of Maturation*

Hormonal manipulation of spawning may be necessary when the duration of gamete collection from a brood stock is abnormally prolonged. Asynchrony of maturation between the sexes also may be displayed by domesticated brood fish. For example, the gamete stripping season for some Norwegian Atlantic salmon brood stocks may extend for periods of more than three months because hatcheries derive their fish from different rivers (Taranger et al. 1992). Of course, experience is the key to determining what manipulation is required for a particular brood stock. All handling of brood stock invokes some level of stress and such stress should be avoided if possible. If good brood stock management practices are not sufficient to overcome site-specific deficiencies in the maturation sequence, manipulation of the physiological processes of reproduction may be the only option.

Greater uniformity in spawning time reduces the sorting and handling of brood stock required to determine their maturity. Corresponding reduction in handling stress leads to improvement in egg and sperm quality. Gonadotropic hormone-releasing hormone (GnRH; *see* Chapter 17), a substance produced by the brain of all vertebrate animals, regulates production and release of gonadotropic hormone (gonadotropin, GtH) from the fish pituitary gland. The pituitary gland in turn regulates development and maturation of the gonads.

Following identification of the hormone structure of GnRH by Schally and Guilliman in 1972 (reviewed by Schally et al. 1976), several types of superactive GnRH peptide analogues (GnRHa, also called LHRHa) were produced and some analogues have demonstrated remarkable bioactivity in fish. These analogues have been successful in stimulating release of GtH (Crim et al. 1987). GnRHa treatment of salmonid brood stock, including mature female rainbow trout (Crim et al. 1983; Sower et al. 1984), female coho salmon (Sower et al. 1982; van der Kraak et al. 1985, 1987) and female Atlantic salmon (Crim et al. 1986), increased plasma GtH levels and advanced ovulation, thereby synchronizing and reducing their spawning period. However, brood fish must be well advanced in the maturation cycle before applying the hormone treatment (Crim et al. 1986). Premature ovulation reduces egg quality. In more recent tests on synchronizing ovulation in Atlantic salmon brood held in brackish seawater, Taranger et al. (1992) examined a range (1-100 μg/kg) of GnRHa doses and the influence of single vs. double GnRHa (des Gly$^{10}$[D-Ala$^{6}$]LHRH-ethylamide) injections. By withholding hormone treatment until just a month before spawning, a single 10-μg/kg injection of GnRHa is sufficient to accelerate and synchronize ovulation in Atlantic salmon. Higher doses of GnRHa (e.g., 100 μg/kg) reduce egg quality (L. Crim, unpubl. data).

### *Altering Photoperiod to Produce Eggs Throughout the Year*

Supplying the market with fish throughout the year, or when optimum market prices are anticipated, requires control of the timing of production of egg batches and therefore fry supplies. Although seasonal breeding habits of wild salmonids are normally restricted to specific times of the year, selecting different strains of fish with early or late natural spawning times can spread the maturation time and expand the egg production period by several weeks. When these differences in natural spawning times are combined with advancement or delay of the rate of maturation by photoperiod manipulation (*see* review by Bromage et al. 1992), spawning and egg production can be achieved throughout the year. For example, long days early in the reproductive cycle can advance maturation while short days during the initial few months lead to delays in development of the gonads. Relatively simple and inexpensive equipment is required to control reproduction by means of artificial manipulation of photoperiod. In this context, Ingram (1985) recommended using automatic timer switches to achieve constant and accurate changes in day length. He also suggested that normal light bulbs be used for artificial lighting but cautioned that the dark side of the photoperiod should be completely dark because salmonids have a very low threshold to light.

## CONCLUSIONS

Effective broodstock strategies can be developed from existing knowledge bases, particularly when management objectives are clearly defined and matched with sound scientific principles. Scandinavian countries, with their structured salmonid breeding programs (Nævdal 1981, 1991; Refstie 1990), have produced significant national benefits to Norway and all of northern Europe. This example reveals how much can be achieved. The world's economic and environmental pressures are persuasive forces toward understanding and dealing with the uncertainties of the biological, business and social milieu of salmonid broodstock management.

Because effective broodstock programs must consider their impact upon natural salmonid populations, current research should be directed at concerns about potential impacts of cultured salmonids on wild populations, especially where aquaculture brood stocks differ genetically from wild populations in the area of a salmonid farm. Hindar et al. (1991) stated that the impact of individuals of distant origin on wild populations can be summarized in three general categories: interbreeding, competition, and contamination (i.e., introduction of pathogens). A considerable amount of work is being focused on this topic (Hindar et al. 1991; Pepper 1991) but there are as yet no definitive conclusions.

The following recommendations of Hindar et al. (1991) are particularly important:

(1) Catalogue genetic characteristics of natural populations that potentially would be genetically affected by aquacultural releases (e.g., allele frequencies, spawning times, life history variables, etc.). This work should be undertaken in parallel with aquacultural activities to provide baseline data for subsequent monitoring.

(2) Undertake a variety of experiments to provide empirical data concerning the genetic effects of aquacultural activities on natural populations. Particularly important are experimental releases of genetically identifiable fish, which should precede any proposed large-scale rearing or release, and the results of which should dictate the scope of such activities.

(3) Encourage rearing of sterile fish for aquaculture through use of sterile hybrids and applications of such procedures as induced triploidy and hormonal sterilization... an understanding of the potential ecological and genetic effects of releasing such individuals should precede any large-scale rearing or release.

This chapter has attempted to provide a compilation of accumulated knowledge pertinent to management of salmonid brood stocks. While there is still much to learn, sufficient knowledge exists to avoid some of the pitfalls of ignorance of this topic. The financial capacity to use this knowledge will be determined not necessarily by the availability of the financial resources, but largely by the extent to which natural and captive stocks deteriorate amid minimal cost programs. It is possible that low-cost programs will continue to support economically viable salmonid aquaculture operations. However, serendipity and economics alone are not rational bases for a broodstock program.

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## Chapter 5

# GAMETE REMOVAL, FERTILIZATION AND INCUBATION

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## INTRODUCTION

### *Scope of Chapter*

This chapter deals with the final part of the process of artificial reproduction, i.e., the collection, handling, management, and preservation of gametes, artificial insemination, and incubation. The quantity and quality of the gametes, and the success of fertilization and incubation are largely dependent on the previous steps of the reproductive processes and particularly gametogenesis, which is strongly influenced by the physiological state and the environment of the brood males and females, the age at maturation (which influences the first reproductive cycle), husbandry, and the state of domestication of the strain. Some of these aspects are detailed in other chapters. In addition, the methodology and influences of important physical, chemical, and biological factors on egg development and survival are discussed.

### *Importance of Gamete Quantity and Quality in Culture Operations*

In salmonid culture, as in any other animal production system, the technical and economical performance of the hatchery depends in large part on the quantity and quality of gametes and the yield of highly viable fry. The objective is to keep the minimum number of brood stock of the required quality in the hatchery and extract all gametes available. This is particularly true for salmonid females which are less fertile (lower fecundity) than many other fish species. A salmonid female yields between 500-2,000 eggs per kilogram of body weight (Bromage and Cumaranatunga 1988) compared to 100,000 to 200,000 in cyprinids (Horvath and Luckowicz 1982). It was demonstrated by Bromage and Cumaranatunga (1988) that in rainbow trout (*Oncorhynchus mykiss*) the relative fecundity depends on the size of the female: 2,000 eggs/kg body weight in a 3-kg female and 4,000 in a 500-g female. It is necessary that the eggs are of a good quality, yielding high quality fry which should meet the specific needs of the hatchery or farm. Many factors determine the quantity and quality of eggs and will be discussed below.

The situation is less critical for the male in which spermatozoa production is in the range of $10^{12}$ spermatozoa per kg of body weight (Billard 1983*a*, 1983*b*), but only a part of this production can be collected and utilized for artificial insemination. However, the main limitation in males is that a minimum number of sires is necessary in the hatchery in order to keep a satisfactory genetic equilibrium in the population (*see* Chapter 11).

### *Major Problems Encountered, Their Frequency of Occurrence and Cost*

Several major problems exist in the procedure of gamete collection, handling and preservation, and fertilization and incubation.

(1) Seasonality of reproduction. There is a great need for spreading the gamete availability throughout the year; marketable fish must be supplied throughout the year, and fry should be constantly available. Importation of eggs from a distant source is not satisfactory because of disease problems and the need to have a well adapted strain on a given fish farm. The use of several strains spawning at different times of year or the use of a strain spawning twice a year, as done in Japan (Aida et al. 1984; Lou et al. 1984), offers a solution to this problem. However, much care is required to hold several strains in the same hatchery and to maintain their genetic integrity to ensure that there is no change in the spawning period. Photoperiod manipulation of breeding cycles is a recent development (Bromage et al. 1992). All of these procedures may result in the maturity period being out of phase between males and females. Hence, good quality sperm may not be available when females ovulate.

(2) Failure to meet environmental requirements of the brood stock. The main factors include temperature, photoperiod, food quality and balanced diet, and water quality. Interactions may be found between some of these factors. For example, photoperiod and thermoperiod were found to influence gamete quality (McQuarrie et al. 1978). Current laboratory observations indicate that spermiating salmonid males in late winter and spring yield poor quality sperm compared to males of the same population spawning in fall and early winter (Billard 1992).

(3) The erratic timing of ovulation in a given population. Females may ovulate over a period of a few months, especially rainbow trout. The control of ovulation, especially the synchronization of ovulation, is advisable so that a given number of females may ovulate each day to facilitate work in large hatcheries.

(4) Low egg quality. The quality of gametes, especially eggs, is a key factor in the success and efficiency of the artificial reproduction procedure. Quality is often highly variable and in some cases is so low that the eggs must be discarded. High quality gametes are easy to handle and to preserve. They yield a higher percentage of fertilization, embryonic development, and hatching. Therefore, less work is required removing dead eggs, and fungus growth on eggs is reduced. Finally, high quality eggs reduce production costs.

(5) The preservation (storage) of gametes *in vitro* after stripping. Eggs should be fertilized soon after collection. Therefore, males and females must be handled in the hatchery simultaneously, making the work more complex. Transportation is also limited and storage methods will depend on the distance and period of time from collection to fertilization (*see* page 319).

(6) Water quality. Losses during incubation may be high if water quality is poor or flow rates inadequate or interrupted due to equipment failure. To ensure optimum water quality conditions, regular monitoring of water flows, dissolved oxygen, pH, and other important factors is necessary. With the advancement of microcomputers and electronics in general, automated monitoring and data-logging of such of factors is now quite common and reasonably inexpensive.

(7) The physical deterioration of spawning salmon. The maturation process for salmonids results in a poor physiological state of both males and females after reproduction. It is responsible for 100% mortality in semelparous species such as Pacific salmon. In iteroparous species, such as Atlantic salmon (*Salmo salar*) and sea-run trout such as steelhead (*Oncorhynchus mykiss*) and cutthroat trout (*O. clarki*), it may be possible to reduce losses by appropriate treatment (i.e., ensuring optimum diets with vitamin supplements and low fish density in holding tanks) of broodstock prior to and after reproduction.

### *Current Research and New Directions*

Several topics have been intensively studied and have yielded practical applications to salmonid culture:

(1) Induced spawning. In wild salmonids, ovulation and spermiation occur simultaneously, but in captivity these two events sometimes do not match and ovulation is often spread out over long periods. Active research has been carried out

recently to synchronize ovulation and stimulate spermiation by appropriate hormonal treatment, sometimes in the form of pellets implanted under the skin (Zohar 1989; Breton et al. 1990) or via oral administration (McLean et al. 1990; Breton 1995). This is based on earlier intensive work on endocrinology of the last part of gametogenesis, especially in females (oocyte maturation and ovulation) (*see* Chapter 17).

(2) Definition of proper diluent. For sperm (especially to improve motility), an important effort has been made in this field recently. The results have helped to define the short duration of motility of salmonid spermatozoa and have demonstrated methods of improving insemination of eggs (Billard 1992).

(3) Sperm cryopreservation. The techniques for cryopreservation are not fully satisfactory but the basic knowledge acquired on the biology of the sperm will probably contribute to the improvement of cryopreservation techniques (Leung 1991; Leung and Jamieson 1991; Billard 1992; Rana 1995). A high rearing temperature (i.e., 18°C) and feeding males with lipid-rich diets during spermatogenesis was shown to improve sperm cryopreservation (Labbé et al. 1991).

(4) Egg preservation. Egg cryopreservation has not been very promising due to the large amount of water in the yolk, which results in ice formation and cell damage during freezing. One approach is to keep eggs or embryos in a non-frozen stage at a temperature slightly below 0°C for short-term preservation (Maisse, unpubl. data, 1992).

(5) Manipulation of eggs and chromosomes. The gametes of salmonid species, both eggs and sperm, are easy to obtain and have a relatively long survival after collection in comparison with other species. This has allowed some manipulation of eggs and chromosomes and even gene transfers. Recent research has been active in this area leading to gynogenesis, polyploidisation, and transgenesis (Depêche and Billard 1994; and *see* Chapter 17). Previous work on gamete manipulation (Billard and Jalabert 1974) had facilitated this work. Diluents were developed for immobilisation of the sperm (allowing dilution of seminal plasma without initiation of motility) so that sperm could be exposed to ultra-violet (UV) irradiation, for instance, in the case of gynogenetic activation (Quillet 1994).

(6) The role of social interaction. The stimulation of reproductive behaviour elicited by pheromones has been studied in salmonids as in other groups of fish (Büyükhatipoglu and Holtz 1984; Liley and Rouger 1990) and may create the possibility of regulation of ovulation, spermiation, and even earlier stages of gametogenesis, maturation and sexual differentiation.

Some other fields as follows, have been less well studied:

(1) Gametogenesis and gamete aging. The importance of certain substances such as nucleotides, hormones, and steroids, laid down in the eggs during oogenesis is not well understood (Billard 1992). The process of gamete aging both *in vivo* (spermatozoa free in testicular lobules or eggs after ovulation) and *in vitro* storage after stripping should be studied for Atlantic salmon and sea-run trout species to determine windows of opportunity for obtaining optimum gamete quality.

(2) The process of fertilization. Sperm penetration, mechanisms involved in the fusion of pronuclei, modification of the cytoskeleton, energy requirements of gametes, and changes in membrane permeability are all inadequately known.

(3) Water hardening. The process of water hardening is not well understood. The hardening of the egg capsule is essential for mechanical resistance and protection of the eggs against the hypo-osmotic environment during embryogenesis.

(4) Molecular and genetic aspects of embryonic development. The early stages of development have been widely studied from a morphological point of view, but the molecular aspects of gene expression are now less well studied in salmonids in comparison with other fish species such as medaka (*Oryzias latipes*) or zebrafish (*Brachydanio rerio*) (Depêche and Billard 1994).

## DETERMINATION OF FINAL MATURATION IN MALE AND FEMALE STOCKS

### *Physiological Description*

*General Physiological Stage.* The physiological state of the fish when entering into the final stages of maturation and spawning is highly variable, and is related to a large variety of factors including the nature of the species. Pacific salmon show progressive degradation and die after the first spawning. Iteroparous species, such as rainbow trout, have a lower level of insulin suggesting a reduction of energy stores preceding mortality (Plisetskaya et al. 1987). Although there is considerable degradation of the physiological state with a marked endocrine imbalance, the quality of the gametes remains satisfactory. In contrast to these, other parameters which influence the physiology of broodstock such as husbandry, feeding, age, and environmental effects, have a strong impact on the quantity and quality of gametes. In addition, unusual occurrences have been reported, such as coho salmon (*Oncorhynchus kisutch*) exhibiting a dysfunction in androgen and cortisol secretion in a population in Lake Erie, which was related to low fecundity and a poor expression of secondary sexual characteristics (Morrison et al. 1985).

Rearing conditions are also key factors in reproduction. For example, insufficient feeding at a specific period may prevent maturation in Atlantic salmon males and females (Rowe and Thorpe 1990; Thorpe et al. 1990). In the brook trout (*Salvelinus fontinalis*), ovulation is significantly delayed when females are reared in acidic water (pH 5.16-4.48) (Tam and Payson 1986). More generally, mature fish are fragile and sensitive to stress and diseases. Pohl-Branscheid and Holtz (1990) reported physiological exhaustion of fish submitted to repeated foreshortened light cycle due to increased frequency of handling and stripping, especially in males.

*Reproductive State of Pre-spawning Fish.* A summary of oocyte developmental stages of rainbow trout (applicable for all salmonid species) is outlined in Table 1 (after Bromage and Cumaranatunga 1988). During vitellogenesis there is a massive accumulation of yolk in salmon eggs, a further increase in the zona radiata, and development of rough endoplasmic reticulum in the granulosa and thecal cells (stage 5) (Bromage et al. 1992). At the end of vitellogenesis there is a progressive migration of the nucleus from the centre of the oocyte to the periphery (stage 6). At this time the yolk globules coalesce, especially in the centre of the cell. By the end of stage 6 the nucleus (germinal vesicle) is close to the oocyte membrane. The final movement of the germinal vesicle (which eventually touches the plasma membrane) and the rupture of the nuclear membrane is termed germinal vesicle breakdown (stage 7). When the germinal vesicle moves close to the micropyle at the animal pole of the oocyte, the female pronucleus is located near the point of penetration of the spermatozoan. Ovulation occurs shortly after oocyte maturation and results from the rupture of the follicular wall. At the same time the micropylar cell, which blocks the micropyle, is removed so that the spermatozoan can penetrate through the egg capsule after oviposition. The regulation of maturation and ovulation has been studied for several species of salmonids including rainbow trout (Jalabert et al. 1973; Jalabert 1976) and brook trout (Goetz and Bergman 1978; Cetta and Goetz 1982). At the end of vitellogenesis a complex endocrine change occurs with the appearance of $17\alpha$-hydroxy-$20\beta$-dihydroprogesterone, which is involved in oocyte maturation and in the synchronization of maturational and behavioral events during spawning (Liley and Rouger 1990).

At the end of spermiogenesis, the spermatozoa are released from the cysts into the lobular lumen. However, opening of the cysts may occur earlier and some spermatids may be seen in the lumen. Progressively the lumen becomes full of spermatozoa and no other germ cell stages are present except A-type spermatogonia (GA), which will be the starting point for the next cycle. The spermatozoa remain in the lobule for about one month in an apparently quiescent state although they may undergo some maturation (*see* below). Some of them reach the genital tract and may be extracted by firm abdominal pressure. The full process of spermiation occurs under hormonal stimulation, which induces a strong secretion of fluid (hydration) in the lobule. This event is thought to increase the interlobular pressure, pushing the spermatozoa into the sperm duct (Billard 1986) in which active ion transport occurs (Marshall et al. 1989). The hormonal environment in the testis when spermiation is initiated also determines the capacity of motility of spermatozoa (Nagahama 1992).

TABLE 1

Summary of the stages of oocyte growth of rainbow trout based on the changes occurring in the nucleus and the cytoplasm, especially the yolk (after Bromage and Cumaranatunga 1988). Stages are applicable to all other salmonid species.

| Main divisions | Stage no. | Morphological characteristics | Sub-stages | Cell diameter ($\mu$m) | Occurrence (months after hatching) |
|---|---|---|---|---|---|
| Primary growth (previtellogenesis) | 1 | Chromatin nucleolar | | 20-50 | 6 |
| | 2 | Early perinucleolar with balbania bodies (bb) | a) basophilic cytoplasm<br>b) bb near the nucleus<br>c) bb at the periphery | 70<br>250 | 8 |
| | 3 | Late perinucleolar | | 300-400 | 9-10 |
| Yolk vesicle (endogenous vitellogenesis) | 4 | Vesicle | a) vesicle at the periphery<br>b) vesicle in the whole cytoplasm | 500 | 12 |
| True vitellogenesis | 5 | Peripheral yolk granules | | 2,000-3,000 | 15-18[a] |
| | 6 | Yolk granule migration | | 3,000-5,000 | |
| Maturation/ ovulation (hydration) | 7 | Germinal vesicle breakdown | | 3,500-6,000 | 24[a] |

[a] Only for females maturing at 2 years of age.

*Quantitative Aspects of Gamete Production.* In females the quantity of gametes, or fecundity, is expressed by the number of mature follicles (potential fecundity) or by the number of ovulated oocytes per female (absolute fecundity); or as eggs per kg of body weight (relative fecundity). Fecundity is influenced by a large number of factors.

Young females at their first reproductive cycle yield a lower number of eggs than older females but the relative fecundity (eggs per kg body weight) is often higher. Fecundity depends both on body size and egg size; egg size varies greatly with species, the Pacific salmon often having the largest eggs (4-8 mm or more) and the trout and charrs often having smaller eggs (ca 3-5 mm) (*see also* Chapters 2 and 3).

In Pacific salmon egg size tends to increase with age and growth of females (Kaev and Kaeva 1987). The feeding regime of the female and the quality of feed (Eskelinen 1989), the growth rate of the female (Takashima 1986), the genotypic background (Bromage et al. 1990), and the body weight (Huang and Gall 1990) affect the size of eggs and fecundity in various salmonid species. In chum salmon (*Oncorhynchus keta*), egg survival was different among families and was higher in small females than in large ones (Beacham and Murray 1985). In Atlantic salmon of the Neva River (former USSR), the size and weight of eggs increase with the time fish spend in the sea (Kazakov 1981*a*). In Norway the size of eggs was found to be different in females taken from different rivers (5.5 ± 0.16 mm to 6.6 ± 0.14 mm) (Aulstad and Gjedrem 1973). In rainbow trout, the presence of high levels of carbohydrates and low or intermediate levels of protein in the diet increases the survival rate of eggs and fry compared to high protein levels and low carbohydrates (Shayne Washburn et al. 1990). More specifically an increase in temperature was shown to raise the vittelogenin concentration in serum in Atlantic salmon (Olin and von der Decken 1989) and the size of the eggs in rainbow trout (Billard and Breton 1977). Post-spawning egg retention is usually limited and is estimated at 6% by Manzer and Miki (1986). For more details on the quantitative aspects of reproduction, see Billard (1987) and Bromage et al. (1992).

The production of male gametes (i.e., spermatozoa) is very high in salmonids, in the range of $5\text{-}8 \times 10^{12}$ spermatozoa per kg body weight per year. This is among the highest sperm production levels recorded for teleost fish (Billard 1987). At the end of spermatogenesis, the spermatozoa concentration is $5.5 \times 10^{10}$ per gram of testes in Atlantic salmon (Kazakov 1981*a*); $2.5 \times 10^{10}$ in brown trout (*Salmo trutta*) (Billard 1983*b*) and $5.8 \times 10^{10}$ in rainbow trout (Billard et al. 1971). Sperm production is lower in young males of Atlantic salmon — $4.4 \times 10^{11}$ spermatozoa per kg body weight (Billard 1974). A similar observation has been made on brown trout (Billard 1974).

However only a part of this sperm production can be used for fertilization because all spermatozoa are not released during the period of spermiation (Billard 1986). In rainbow trout a spermiation yield of 22% was reported by Billard et al. (1971). However these fish were transferred from a fish farm to the laboratory after the initiation of spermiation and the males were probably stressed. A poor yield of sperm can be deduced from the work of Kazakov (1981*b*) on the Atlantic salmon since the testis weight did not change during the spermiation period. Munkittrick and Moccia (1987) reported a collection of 2.5 x $10^{11}$ spermatozoa per kg of body weight during a stripping period from 14 to 25 weeks, which is less than 4% of the total amount of spermatozoa in the testes. Better yields have been reported, for instance 47 ± 7%, in the case of rainbow trout after their first reproductive cycle (Billard 1974). Current observations show that at the end of the spermiation period, rainbow trout males may have empty testes. In such instances it is assumed that most of the spermatozoa have been collected. However, they may have been resorbed in the testes as spermatozoa phagocytosis has been reported soon after the spawning period (Billard et al. 1972). There is a large variation in the yield of spermatozoa between males and among populations as shown by Baynes and Scott (1985); the percentage of spermiating males and the volume of milt (i.e., the mixture of seminal plasma and sperm) collected were different in three strains of rainbow trout. Gjerde (1984) reported that in 70 one-year-old male trout sampled three times at the peak of the spawning season, no semen was obtained from 4, 9, and 7 males at the first, second, and third stripping, respectively. The duration of spermiation is also variable and has been reported to last more than four (Gjerde 1984), 10 (Billard 1974), and up to 25 weeks (Büyükhatipoglu 1977, quoted by Gjerde 1984).

The spermatozoa concentration in milt is in the range of 5 to 15 × $10^9$ spermatozoa per ml. It varies throughout the reproductive season and decreases with age (Khorevin 1988). Sometimes the duration of spermiation is shorter than the period of ovulation so that a shortage of sperm may occur at the end of the season, but usually the duration of spermiation exceeds that of ovulation. In that case all of the sperm production cannot be utilized. Khorevin (1988) noted that chum salmon males participate in spawning for only a very short time.

### *Importance of Gamete Quality in Culture Operations*

The quality of gametes is discussed in more detail in this section. Quality is usually assessed by the percentage of fertilization, reflecting both the fertilizing capacity of the spermatozoa and the capacity of the egg to be fertilized. However, this gives only a rough estimate of quality since there is interaction between the quality of both gametes; poor sperm quality does not allow an estimate of egg quality and vice versa. In addition, the ratio of spermatozoa used to eggs in a known volume of diluent often is overlooked. To evaluate the true fertilizing capacity of spermatozoa, fertilization tests should be carried out over an increasing dilution rate such as $10^{-2}$ to $10^{-4}$ or in some cases up to $10^{-6}$ (Billard 1975) (Figure 1). Other criteria specific for either gametes should also be used in addition to fertilization tests to evaluate gamete quality. For example, fish culturists assess the "ripeness" (proportion of eggs ovulated) of eggs, discarding eggs that are still attached to the skein or eggs that are overripe or water-hardened. If the ovarian fluid with the eggs appears cloudy or milky, usually indicative of bacterial infection, egg quality should be considered poor.

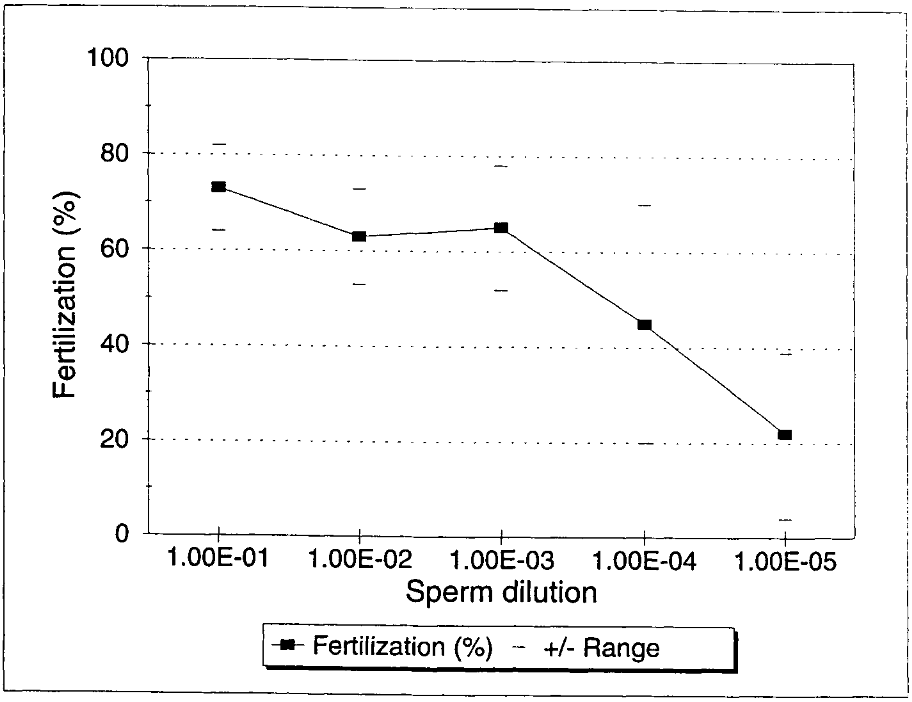

FIGURE 1. Changes in fertilizing capacity (% ± range, n = 14) of rainbow trout sperm using increasing dilution rates ($10^{-1}$ to $10^{-5}$) (from Yazbeck-Chemayel 1974).

Objective evaluation of egg quality is difficult. The strength of the egg capsule has been proposed by Winnicki and Bartel (1967). Some observations of this structure are possible after fixation (Fleming 1987). Kato and Kamler (1983) made various measurements on rainbow trout eggs (size, water content, calorimetry, chemical composition) which were not always correlated with survival during embryogenesis and hatching. Egg quality shows high inter-female variability (Fredrich 1981; Craik and Harvey 1986) and may be related to other factors such as chemical composition, age of female, size, time of stripping, and various factors of husbandry (Bromage and Cumaranatunga 1988; Springate et al. 1985). Craik and Harvey (1984) found that the main factor affecting fertility was the interval between ovulation and stripping. In carp (*Cyprinus carpio*), Boulekbache et al. (1989) related the decline of post-ovulatory fertility of eggs with a decline in egg ATP content; it is assumed that accumulated energy in the egg is required for activation, fertilization, and first cell division. This relationship should be investigated in salmonids. Thyroxine is found in eggs (Kobuke et al. 1987; Tagawa and Hirano 1987). The addition of triiodothyronine to the eggs of striped bass (*Morone saxatilis*) by injecting the female before ovulation was shown to increase embryo and fry survival (Brown et al. 1989). However, this was not confirmed in salmonids by Mylonas et al. (1991). The amount of carotenoid in the egg has no clear effect on the hatching rate (Craik 1985); it may even sensitize the eggs to light (Torrissen 1983). Ascorbic acid content in the egg also has been related to hatchability of embryos (Dabrowski and Blom 1994).

The age of females and their growth rate are also reported as factors determining the quality of eggs. In Atlantic salmon Tikenko (1985) showed that females with a high growth rate after two years at sea yielded a better survival rate of their progeny than slowly growing females of the same age. Beacham and Murray (1985) found that chum salmon egg survival from fertilization to hatching was highest for eggs from small females and lowest for eggs from large females. This was observed at three different incubation temperatures, 4, 8 and 12°C.

The environment of the female is also important. Water salinity during the prespawning period strongly affects egg quality. This was shown in Atlantic salmon, where egg survival improved with a period of freshwater residency of several weeks prior to spawning, compared to brood fish held in seawater until spawning (Craik and Harvey 1986). In chum salmon, the fertilization rate is significantly decreased when females are held in seawater compared to females placed in fresh water for the last part of maturation (Lam et al. 1982; Stoss and Fagerlund 1982, 1985). It is clear that the final stages of maturation are accompanied with a loss of osmoregulatory ability in seawater, at least for Pacific salmon. However, careful handling of broodstock in seawater does allow a successful yield of gametes (Lam et al. 1982)

Ketola (1983) found that mineral supplement in the diet of Atlantic salmon females had no effect on egg quality. In contrast, micropollutants accumulated in oocytes during vittelogenesis were found to influence egg quality (Craik and Harvey 1988). Coelomic fluid secreted in the body cavity contains constituents originating from the maternal circulatory system (Matsubara et al. 1987) and contains up to 20 mg of protein per ml, with a diversity of animo acids protecting the egg (Billard 1983*b*). However, if its composition is impaired, this protection may be lost and eggs may become activated and water hardened inside the body cavity (Lam et al. 1982). The quality of eggs left in the body cavity after ovulation declines progressively and they will become overripe within a time period, which is dependent on age of female, water temperature, and species. In rainbow trout, egg survival after ovulation has been observed for a period of weeks (Escaffre et al. 1977). Billard and Breton (1977) reported that *in vivo* survival of eggs is much shorter at 20°C than at 10°C.

The quality of sperm is usually assessed by the intensity of motility, as a percentage of motile spermatozoa and duration of forward movement (Goryczko and Tomasik 1975; Sanchez-Rodriguez and Billard 1977). This percentage is a valid parameter provided that the dilution rate is adequate (>1:1000) in order to allow a good estimate under the microscope and to initiate motility synchronously (Billard and Cosson

1990*a*, 1990*b*). Another sperm-motility method using quasi-elastic-light scattering (Craig et al. 1983) did not give reliable results. Motility can also be assessed by stroboscopy (Cosson et al. 1985; Billard and Cosson 1989). Other sperm quality assessment parameters include swelling of the plasma membrane (Winniki 1967), spermatocrit (i.e., percentage packed cell volume in seminal plasma), or the chemical composition of the seminal plasma (Piironen and Hyvärinen 1983; Billard and Menezo 1984; Schmehl et al. 1987), including ascorbic acid content (Ciereszko and Dabrowski 1995). Degradation of the spermatozoan membrane results in the release of a cellular compound in the seminal plasma that can be measured as a quality test. For instance, compounds such as lactate dehydrogenase (Gallant and McNiven 1989; McNiven et al. 1992) or plasma membrane proteins (Maisse et al. 1988) have been measured.

The quality of spermatozoa changes during the reproductive period; the inhibitory action of $K^+$ varies (e.g., Figure 3), especially at the beginning and the end of the period (Billard and Cosson 1990*a*). The intensity of motility declines slightly as well as the fertilizing capacity (Yazbeck-Chemayel 1975; Moccia and Munkittrik 1987). The quality of sperm also varies over various portions of the male genital tract (Billard 1976; Morisawa and Morisawa 1990).

Under some conditions, it is possible to relate sperm motility with its fertilizing capacity but the dilution rate should be high enough to avoid an excess of sperm (Billard 1983*b*). In addition, an excess of non-motile sperm (>90%) should be avoided since this was shown to reduce the fertilization rate (Levanduski and Cloud 1988).

### *The Appearance of Fully Mature Fish and Methods to Verify Maturation*

Evaluation of the state of maturity of broodstock may be based on both objective and subjective parameters. One common parameter used in the laboratory and sometimes on fish farms is the position of the germinal vesicle in the oocyte (*see* above) (Jalabert et al. 1973). The follicles are extracted through the genital pore by pinching the posterior part of the ovary and are examined under a dissecting microscope. The germinal vesicle can be identified through the follicular cell layer when it reaches the periphery of the oocyte. During this stage (peripheral germinal vesicle), the vesicle is clearly visible near the oocyte border. Just before ovulation (end of oocyte maturation) the germinal vesicle breaks down and disappears. This indicates that the process of maturation is over and that ovulation will occur within a few days depending on temperature and species. The closer the vesicle is to the periphery, the sooner ovulation will be. A scale of maturity has been proposed by Cetta and Goetz (1982) in brook trout during the preovulatory period. It is possible at the stage of peripheral germinal vesicle to give acute hormonal treatment to synchronize ovulation (Figure 2). When the germinal vesicle is not visible, the female is far from full maturity and may still be at the stage of yolk accumulation (vitellogenesis). When the follicle is small, less than the usual size of eggs for a given species, vitellogenesis may be in progress, but since there is a

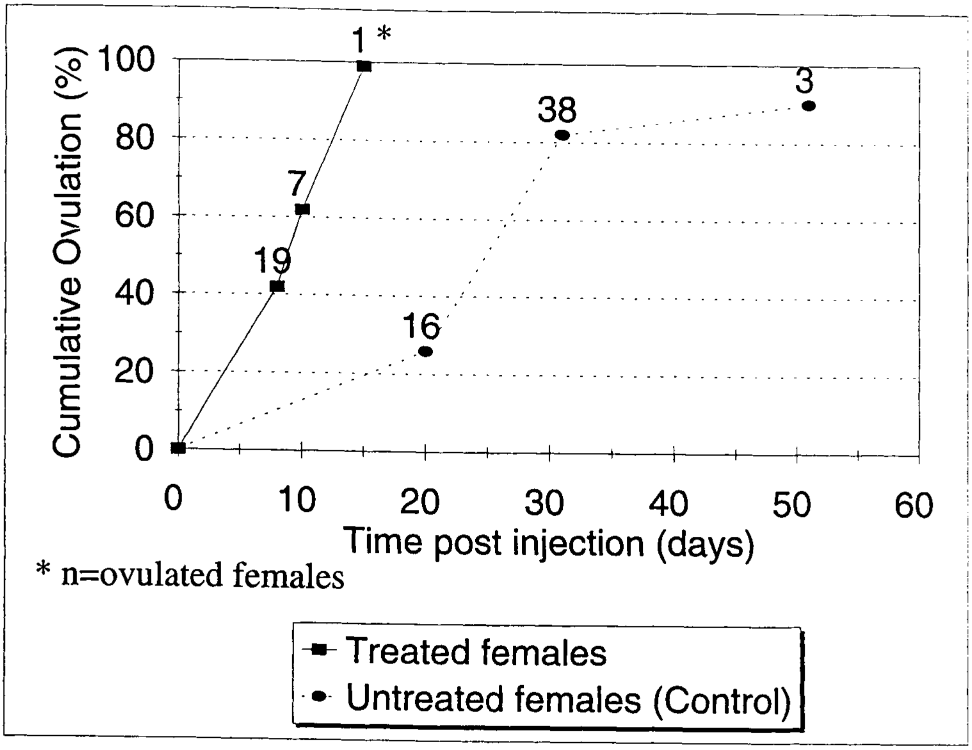

FIGURE 2. Synchronization of ovulation in brown trout after injection of crude pituitary extract (from Billard 1992).

large variation in egg size within a species or strain, this is not a reliable indicator of stage of maturity. The level of circulating vitellogenin has been used to predict whether Atlantic salmon will mature in the year of capture (Idler et al. 1981). When the germinal vesicle is not visible, an acute hormonal treatment will not induce ovulation. Instead, hormonal treatment can be administered by implanting a pellet of slow-releasing, low-level hormones such as GnRH (Breton et al. 1990 in rainbow trout; Crim et al.1983 and Weil and Crim 1983 in Atlantic salmon; and *see* Chapter 4).

More subjective parameters are often used by fish farmers to predict the proximity of ovulation. These include the size and softness of the abdomen, and turgor and preeminence of the genital papilla.

In males, the secondary sexual characteristics such as dark or red colouring of the skin and hooking of the upper or lower jaws are sometimes used to indicate spermiation. Spermiation is usually progressive so that a small amount of milt may be obtained by expression of the posterior abdomen, although at first there will be only a small amount produced. This may be used if necessary although its quality must be checked. The physiology of this "early" sperm is slightly different than for sperm collected later (when yield exceeds several ml/kg body weight). For example, in rainbow trout these early sperm were not immotile after dilution in a 40 mM KCL solution as would have been expected in normal sperm. This motility-inhibition did not occur until sperm were exposed to a level of 60-80 mM KCL (Figure 3), and it is not known if factors other than $K^+$ are involved in the inhibition of motility. This phenomenon of "resistance" to $K^+$, in which $K^+$ does not maintain inactivity of sperm (which may be counteracted by $Ca^{++}$) should be known to fish culturists practicing gynogenesis using an immobilizing solution for sperm prior to UV irradiation and DNA inactivation. Before spermiation starts, spermatozoa in the testes cannot be activated (they have not spermiated) but sperm motility may be improved by the addition of 1 to 5 mM $Ca^{++}$ in the diluting solution as is done for sperm extracted from testes (*see* above). Males in a stage of pre-spermiation may be given hormone injections (e.g., gonadotropin or GnRH) to induce earlier full spermiation (Billard et al.1984; Teranishi et al.1986), yielding mature spermatozoa (*see* above).

## GAMETE REMOVAL

### *Handling Broodstock*

Brood fish are generally of a large size and handling is facilitated by anaesthesia (*see* Chapter 4 for procedure). In cases where broodstock will be kept for future spawnings, special care is required during handling. Semelparous species (Pacific salmon) are usually killed before stripping, yet these fish should also be handled with care during capture or examination. It is essential to avoid wounds on which fungus will rapidly develop. The use of dip nets with rough material or with sharp metal parts should be avoided. Ideally fish should be kept in water while handled, for example in a deep net with a long pouch of impermeable material. When out of the water, fish should be kept on a wet support (towelling or polyurethane foam). Drying fish with a dry towel should be avoided as this removes mucus and abrades skin. It is advisable to keep fish at or below the raceway water temperature. For more details on handling fish, see Leitritz and Lewis (1976), Billard (1990) and Chapter 4.

### *Egg Removal*

Although routinely practiced without apparent difficulty, egg taking is a critical step in artificial reproduction. The membrane of the egg offers some resistance to mechanical pressure, which varies with age of female (Nomura et al. 1974), egg size, and husbandry prior to ovulation (especially feeding). The resistance of egg membranes to

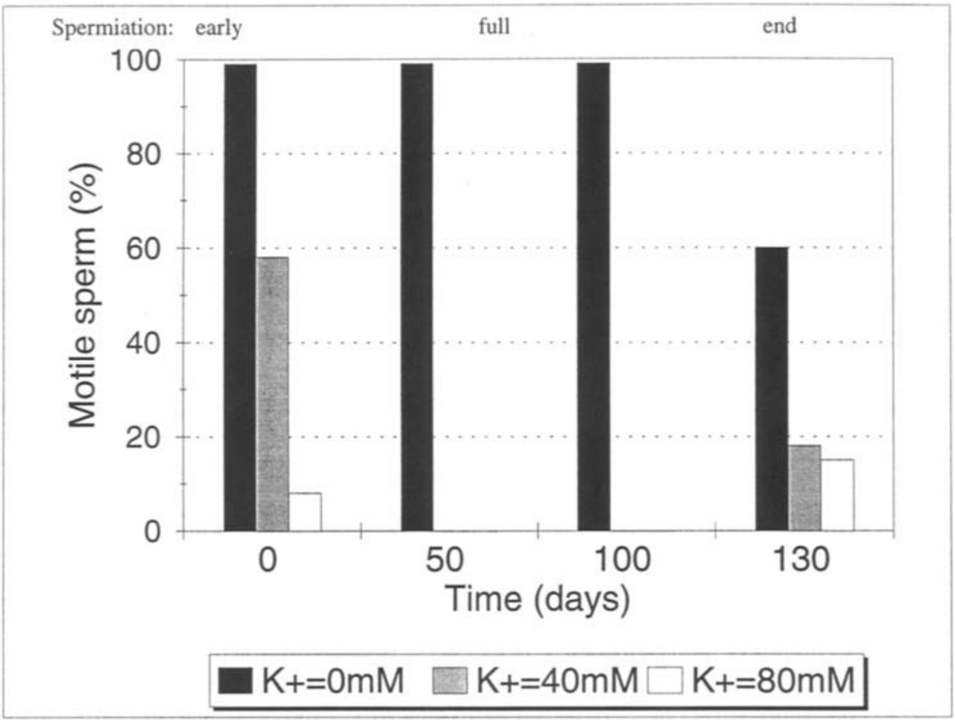

FIGURE 3. Change in sperm motility after dilution in $K^+$ solutions during the spermiation period of rainbow trout (after Billard and Cosson 1992).

mechanical pressure has been evaluated by Winnicki and Bartel (1967), Winnicki et al. (1968), Alderdice et al. (1984), and Jensen and Alderdice (1989). The egg membrane is more fragile in unfertilized eggs than in fertilized and hardened eggs. The plasticity of unfertilized eggs is greater than in hardened eggs (Winnicki and Bartel 1968). Therefore, the membrane may be easily broken by pressure during stripping, especially in younger females which have more fragile eggs. In this case the spawn will become contaminated by yolk, which reduces fertility; when water is added as a diluent, yolk precipitates to form a network which traps spermatozoa and clogs the micropyle (Figure 4). This phenomenon is prevented when a saline solution is used as a diluent for artificial insemination (Nomura 1964; Billard 1977*a*; Wilcox et al. 1984). A rinse of eggs with 14‰ bicarbonate of soda before addition of sperm will also eliminate this problem (Wilcox et al. 1984).

*Egg Taking by Incision.* In Pacific salmon, eggs are removed by incision from killed females. This reduces broken eggs and facilitates egg takes in these semelparous species.

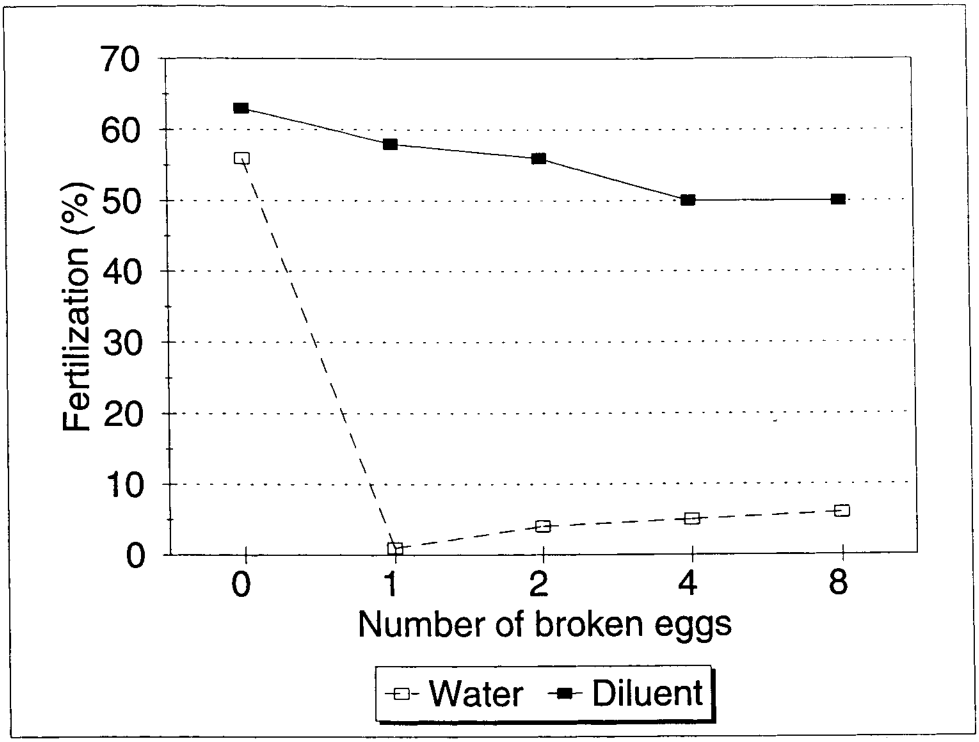

FIGURE 4. The effect of broken eggs (% broken eggs) on egg fertilization in water (W) or saline diluent D532 (125 mM NaCl, 20 mM Tris, 30 mM glycine) (from Billard 1977*b*).

Bleeding, by cutting the gill arches or caudal peduncle, is usually done to reduce levels of blood in the gametes [although blood itself does not impede fertilization (Jensen 1975; Lam 1984)]. Special curved spawning knives are used to cut along the abdominal wall without cutting into eggs which are free in the abdominal cavity. Eggs then spill out into a basin. Eggs that do not fall freely from the cavity may not be ovulated and should be left in the fish. Eggs may survive in the body cavity of dead females for minutes to hours depending on temperature (Billard et al. 1981). See Leitritz and Lewis (1976) and Piper et al. (1982) for more details.

*Egg taking by expression.* The most common technique for egg removal in salmonids, as in many other fish, is stripping by the application of hand pressure to express eggs through the genital pore (Figure 5). One person may do this, but with larger fish, two may be required, one to hold the fish and the other the egg pan.

An alternative technique is to insert a needle under a pelvic fin and blow compressed air into the body cavity to expel eggs through the genital pore. The air is later expelled by hand. This technique leaves fewer eggs in the fish, produces cleaner eggs (less breakage), and is faster with less injury to the fish. Atlantic salmon have been stripped through use of a water jacket, a hollow cylinder of waterproof fabric surrounding the female fish to be spawned. This can be filled with water producing enough gentle pressure on the fish to expel eggs (Piper et al. 1982).

Saline flushes also have been used. An isotonic fluid is injected gently through the genital pore into the coelomic cavity. Then gentle expression forces this liquid and the eggs out through the pore. Employing this technique results in less handling of the fish and less likelihood of damage to the fish. Also, there appears to be less egg retention. The egg is not activated by the fluid.

### *Sperm Removal*

The most common method for sperm removal is to handle the fish as described for the female (Figure 5) and to manually express the milt into a separate container or directly onto the eggs in an egg collecting pan. Collected milt may be held in a tube or beaker and stored on ice until used. The male may also be held on its side on damp sponge material and stripped (Figure 6). Incision is an exceptional procedure for males. When a male does not yield a large amount of sperm, it may be tempting to remove the testes and extract spermatozoa, but these spermatozoa may be of low quality. Spermatozoa are of acceptable quality only after completion of spermatogenesis (i.e., spermatozoa free in the lumen of the lobules). This occurs a few weeks to one month before full spermiation (*see* above). Motility must be checked before fertilization, and, if it is low, it may be stimulated by an addition of 1 mM $Ca^{++}$ to the diluent for insemination. At the end of the reproductive period spermiation stops, although there may still be a large amount of spermatozoa in the testis. These are usually of poor quality and motility must be checked. To compensate for a reduced motility rate, the volume of milt may be increased over the normal ratio of milt to eggs.

### *Criteria for Rejection of Gametes*

There is no objective means of determining the viability of eggs. The general indication is that eggs with heterogeneous yolk must be discarded. Unfertilized eggs that may become white after immersion in fresh water are also of poor quality (normal eggs maintain normal appearance under these conditions for several days or weeks after immersion in water). When 30 to 50% of the eggs from one female appear to be of low quality, all eggs from this fish are considered of suspect quality and should be discarded. Usually sperm taken at the end of a spawning period is of lower quality than at the

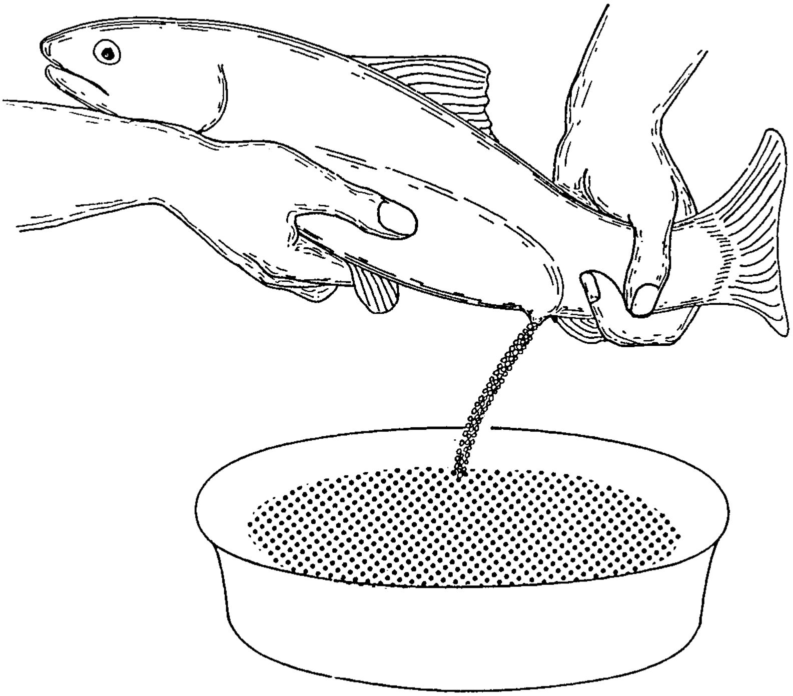

FIGURE 5. Diagram illustrating egg removal by a single person (from Billard 1990).

beginning of the period; late in the spawning period, more sperm will be required for insemination (*see* above).

### *Environmental Conditions Necessary for the Safe Removal of Gametes and Problems Frequently Encountered*

Contamination of eggs and sperm by any material, such as feces, blood, or water, should be avoided during collection and artificial insemination. To avoid contamination by water, fish farmers frequently dry the whole fish, but this results in abrasion of mucus and skin injuries. It is better to dry only the genital area. The risk of water contamination of sperm is slight when the techniques shown in Figure 5 are followed. However, one drop of water into 10 ml of milt would not cause significant sperm activation to be of concern. Furthermore, anaesthetics in the water at usual concentrations do not adversely affect egg fertilization (Billard 1981). The effect of blood

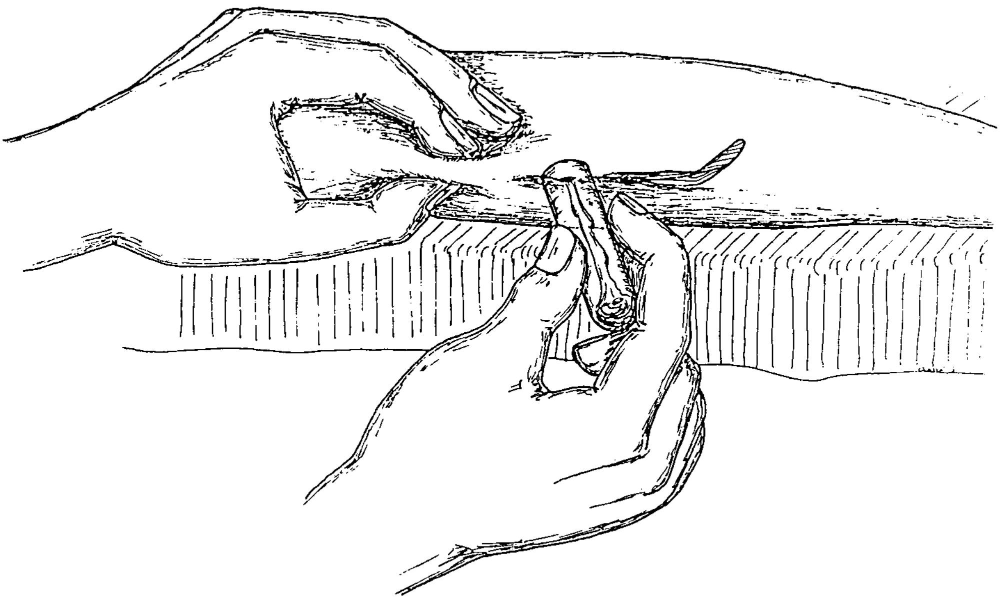

FIGURE 6. Diagram illustrating milt collection in a test tube from a male trout lying on one side (from Billard 1990).

on egg fertilization was studied by Jensen (1975) and Lam (1984), who found that blood contamination up to 12% by volume had no adverse effect on fertilization success.

Thermal shock should be avoided. When gamete removal occurs in winter of fall in a spawning room where temperature does not exceed 10°C, removal and insemination procedures may be done at ambient temperature. In summer, spawning fish exposed to photoperiod manipulation or fish from late or early spawning populations requires more care. As the survival of gametes, especially eggs, is shortened at temperatures of 15-20°C, it is necessary to carry out artificial insemination, or to store gametes at temperatures below 15°C. For rainbow trout and other Pacific salmon in general, fertilization may be done at 15°C but a slight decrease in fertilization is to be expected (Figure 7) and incubation should be carried out at less than 12°C (*see* page 333 for more details on temperature tolerance).

Although there is no clear evidence of harmful effects of light on gametes, it is best not to expose them to direct sunlight. There are some other environmental factors harmful to gametes that should be avoided including chlorine and micropollutants. In Atlantic salmon, the chorion, perivitteline fluid and embryos concentrate cadmium

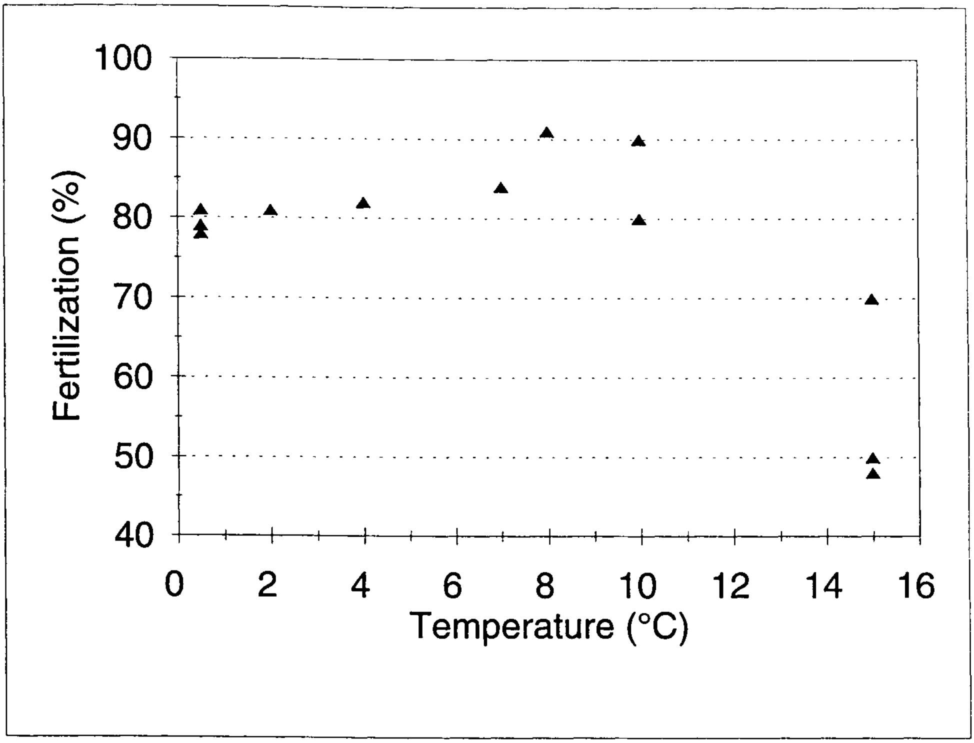

FIGURE 7. Effect of temperature on rainbow trout gametes (acclimated to test temperature prior to fertilization) after fertilization eggs were brought to 12°C and transferred to incubators at that temperature (Billard, unpubl. data).

(Peterson et al. 1985; Peterson and Martin-Robichaud 1986). Exposure of rainbow trout eggs to Lindane (0.25 to 25 ppm) or polychlorinated biphenyls (PCBs, 40 to 400 ppb) resulted in a loss of fertility when eggs were incubated at 20°C but not at 10°C (Billard and Gillet 1981). Diluents with pH levels below 6 and higher than 9.5 have adverse effects on eggs and sperm (Billard et al. 1974; Daye and Glebe 1984).

Gametes should be kept free of mucous, especially when brood fish are infected with fungus, which may later develop on eggs in the incubator. Other pathogens may enter the gametes from fish and cause disease transfers to the next generation; fish with known disease conditions should not be used as brood stock. Iodophor disinfection of newly fertilized eggs is possible and effective in reducing or eliminating most pathogens on the egg surface. Under certain conditions there may some loss of fertility or egg survival (Billard and Montalembert 1976), but disinfection of fertilized eggs during water-hardening with tamed iodine at 100 ppm for 10 min appears to have slight if any negative effects. Eyed eggs may be given the same treatment with no negative effects. Under some circumstances antibiotics have been used to disinfect eggs during water-hardening (Jensen et al. 1981). However, care should be taken to ensure that eggs are not subjected to mechanical shock as a result of changes to standard hatchery practises.

## FERTILIZATION

### *Historical Review of Fertilization Methods*

Artificial insemination in fish was probably first practiced with salmonids. Wilkins (1989) has recently reviewed the history of artificial reproduction of fish, which probably began in the middle ages and was first described by Jacobi in Germany in 1758, and later by several "amateurs" in Britain in 1820-1850 and by Gehlin and Remy in France in 1840-1850. This was the starting point of the considerable development of salmon and trout culture [*see* Kirk (1987) for a historical review of fish culture in seawater]. Further discussions of the history of artificial fertilization are found in Thibault (1989) and Billard (1989)(and see Chapter 1). Several methods of artificial insemination have been used in the past. One was to mix both sperm and eggs with water which gave variable results. Another, called the wet method, consists of mixing first the sperm with water and then adding this mixture to the eggs; this gave even worse results because with a dilution rate greater than 10:1, the spermatozoa would be activated prior to contacting the eggs. The accepted method (known as the dry method) is more successful; sperm and eggs are combined, sometimes with appropriate non-egg-activating diluents, followed by the addition of water (*see* below for details).

### *Review of the Biology of the Fertilization Sequence*

*Activation of the Spermatozoa.* The morphology of the salmonid spermatozoa is simple. The head is spherical and slightly elongated (1.5-2 $\mu$m), the midpiece consists mainly of mitochondria, and the flagellum, type 9+2, possesses bilateral extensions (Billard 1978, 1983*c*) (Figure 8*a*). In chum salmon, Jaana and Yamamoto (1984) have observed a differential distribution of carbohydrate residue in the plasma membrane which may have a role in the process of fertilization. Recent studies on salmonid sperm have been reviewed (Scott and Baynes 1980; Stoss 1983; Morisawa 1985; Billard et al. 1986, 1987; Billard and Cosson 1990*a*, 1990*b*; Perchec et al. 1993). A key feature of the spermatozoan is the short period of motility (less than 30 s in a bufferred saline solution). Duration of motility is even less in fresh water where the cell soon becomes damaged showing disorganization of the cell and eventual rupture of the plasma membrane (Billard and Breton 1970; Billard 1978). In rainbow trout, Hamor (1966) showed that the duration of sperm motility varies inversely with temperature; duration of motility

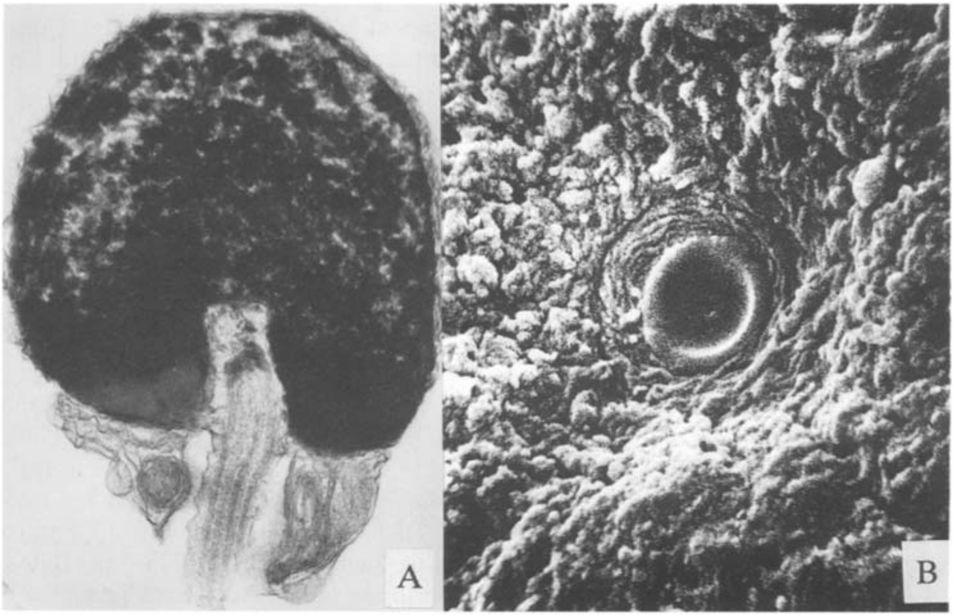

FIGURE 8. Electron micrographs showing (A) morphology of rainbow trout spermatozoa, (B) egg micropyle; opening blocked by material from cortical reaction.

averaged 27.5 and 19 s at 5 and 15°C, respectively. Cosson et al. (1985, 1989) found similar, although slightly higher values of 22 to 25 s at 20°C. These values change according to pH and $Ca^{++}$ concentrations in the diluent (Baynes et al. 1981; Cosson et al. 1989). The frequency of tail beat is most rapid at the start of activation and varies with temperature (Figure 9); the distance of travel is about 3 mm. Initiation of movement is probably due to a change in membrane potential and a reduction of $K^+$ when the seminal fluid is diluted with fresh water; this opens $Ca^{++}$ channels and motility begins (Christen et al. 1987; Tanimoto and Morisawa 1988; Cosson et al. 1989; Gatti et al. 1990; Morisawa and Morisawa 1990). The conditions for activation and maintenance of motility are summarized in Figure 10. The short duration of motility is due to a rapid exhaustion of the intracellular ATP stores, because of the incapacity of the mitochondria to satisfy the high energy demand of the flagellum. The decline of ATP levels has been correlated with the decline in tail beat frequency (Christen et al. 1987; Robataille et al. 1987). However, the mitochondria are still active, since spermatozoa diluted in a saline solution show recovery of ATP level within 15-20 min after dilution, and with the addition of cyanide (CN) at 10 mM, the level of ATP declines. The same is observed when CN is added to non-motile sperm diluted in a 40-mM KCL solution. This shows that the non-motile spermatozoa have a respiratory activity which contributes to maintaining ATP

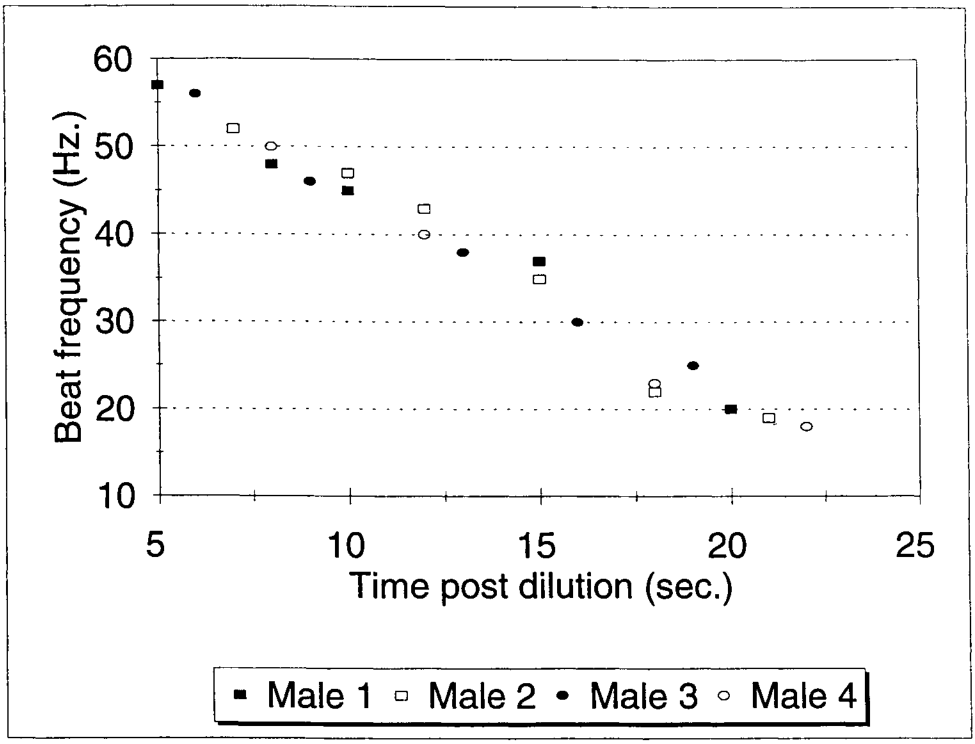

FIGURE 9. Change in the flagellum beat frequency (Hz) of rainbow trout spermatozoa after dilution in a saline solution at 20°C (Billard, unpubl. data).

levels necessary for movement. This strongly suggests that oxygen should be provided during sperm preservation. There are experimental data that show that the fertilizing capacity of sperm, normally maintained for only one day, may be prolonged by the addition of oxygen and antibiotics for up to 30 days (reviewed by Stoss 1983). The better survival of sperm in the sperm duct compared to survival *in vitro* (Marcel et al. 1982) suggests that oxygenation is better in the genital tract, and the often reported low survival of sperm *in vitro* may be due to lack of oxygen leading to exhaustion of ATP and ability to develop motility (*see* below). Motility is also highly dependent on pH. In a well-buffered solution no motility is observed below pH 8, but with addition of $CaCl_2$, some motility is observed at pH 7.5 (Baynes et al. 1981). However in Atlantic salmon, fertilization was reported in acidic water at pH 5 (Daye and Glebe 1984), but this was probably due to the input of coelomic and seminal fluids which contributed to raising the pH at the time of activation.

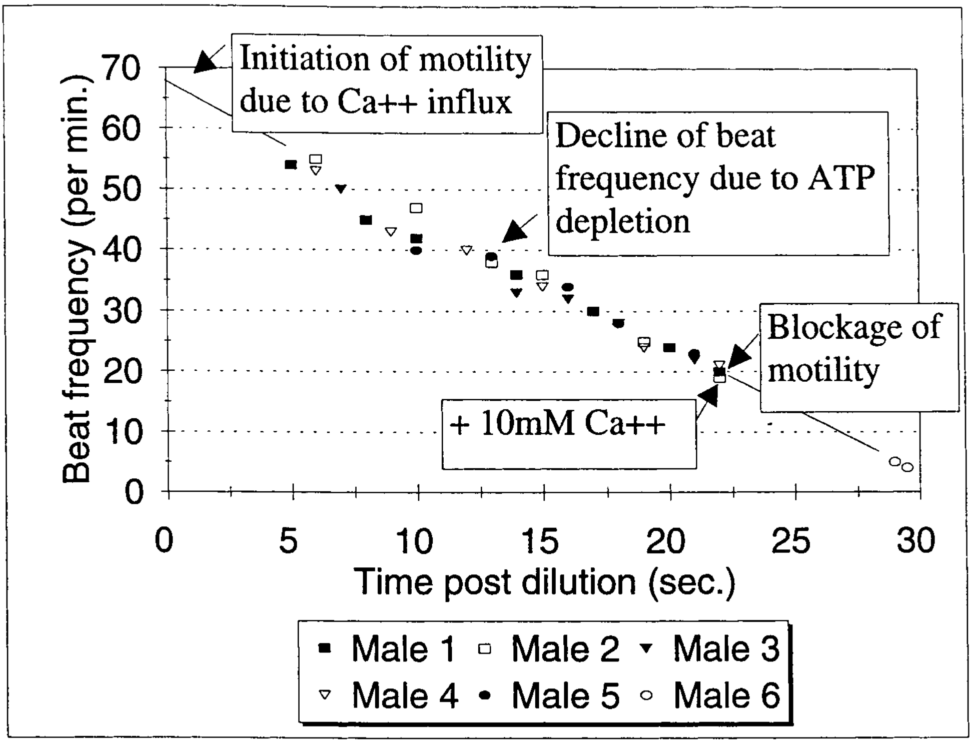

FIGURE 10. Summary of main events occurring during the motile phase of salmonid spermatozoa.

*Biology of the Egg.* As already mentioned, salmonid eggs are large, from 3 to 7 mm depending on species. The egg is surrounded by a thick membrane, the chorion, with a complex lamellar structure that becomes hard after activation. The egg capsule of pink salmon (*Oncorhynchus gorbuscha*) contributes 1% of the total egg diameter (Stehr and Hawkes 1979). This very thick shell cannot be penetrated by spermatozoa which enter the egg through a pore, the micropyle. This brings the spermatozoan very near the female pronucleus. The funnel-shaped canal has an opening of 15-16 $\mu$m and a terminal aperture of 2 $\mu$m in pink salmon (Stehr and Hawkes 1979). A special micropylar cell creates the micropyle and its vestibule through exertion or mechanical pressure during vitellogenesis. This cell undergoes degeneration during the final stages of maturation (Kobayashi and Yamamoto 1985). Immediately after ovulation some cells from the granulosa layer of the theca may remain in contact with the egg, including the micropyle; this may explain why there is a somewhat lower percentage of fertilization just after ovulation than a few hours later. For this reason premature removal of eggs must be avoided.

The chemical composition of eggs (reviewed by Bromage and Cumaranatunga 1988) is variable. Yazbeck-Chemayal (1974) found an energy value of 2.5 ± 0.19 cal/g. Proteins found in unfertilized eggs (Kitajima et al. 1986) become depolymerized after fertilization (Inoue and Inoue 1986). A glycoprotein derived from sialic acid is found in the egg capsule (Inoue et al. 1988), which has bactericidal properties (Kudo and Inoue 1986). Oxygen consumption of unfertilized and nonactivated eggs was found to be 1.29 g/min per egg and did not change appreciably immediately after fertilization and activation (Czihak et al. 1979).

*Activation of Eggs and Fertilization.* A major distinction of the salmonid egg is that the cortical reaction is not induced by fertilization, but is induced by the osmotic shock that occurs when the egg is released into fresh water. Thus the membrane hardening process is independent of fertilization. The breakdown of cortical alveoli is initiated after a release of calcium from the oocyte (Yamamoto 1979). An intake of external calcium is also reported (Khlebovich et al. 1977). The cortical reaction results in water uptake followed by the creation of a fluid-filled perivitelline space, and the hardening of the egg capsule (Hayes 1949; Winniki et al. 1986). During the peri-fertilization stages, the exchange of sodium is confined to the egg capsule and perivitelline fluid (Rudy and Potts 1969). The perivitelline fluid prevents sodium loss from yolk and embryo (Eddy and Talbot 1985).

The release of cortical vesicles into the perivitelline space in chum salmon eggs was studied by Kobayashi (1985) who suggested that the discharge of the vesicle contents was due to the contractile activity of the ooplasm. The discharge of cortical granules is also one step in preventing polyspermy (Ginsburg 1961); material coming from the perivitelline space was shown to block the micropyle and prevent entry of spermatozoa (Szollosi and Billard 1974). Hence, activated eggs are non-fertilizable. Furthermore, Kobayashi and Yamamoto (1981) studied the mechanism of polyspermy prevention and showed that after the sperm head had penetrated into the cytoplasm, no further entry of additional sperm into the cytoplasm was possible even when the breakdown of cortical vesicles was not initiated.

### *Fertilization Protocols*

#### *Immediate Fertilization.*

*1). Ratios of milt to eggs and ovarian fluid.* For maximum fertilization, Scott and Baynes (1980) recommend 1 ml of semen per 1,500-2,000 eggs, based on empirical information obtained from Rutter (1902), Wharton (1957), and Plosila and Keller (1974). Such a ratio is equivalent to about 10 million sperm per egg (Scott and Baynes 1980). Variability in sperm concentration in seminal plasma and sperm and egg quality will have an influence on subsequent egg fertilization. Billard (1992) further discussed the optimum volume of egg to diluted sperm samples for rainbow trout (i.e., 10-20 eggs/ml of diluted sperm, at a ratio of $10^{-3}$ milt:diluent). This translates to roughly 1 or ½ million spermatozoa per egg. This is greater than the 30,000 to 300,000 sperm per egg reported earlier by Billard (1978) or the 75,000 sperm per egg suggested by Erdahl and Graham (1987). Billard (1992) explained the necessity for this large number of spermatozoa required to successfully fertilize a single egg by the small size of the micropyle, the egg diameter (4-6 mm for rainbow trout) and the short trajectory of motile sperm (3 mm).

The use of diluents to ensure optimum fertilization success has been thoroughly studied (Billard 1975, 1977*a*, 1977*b*, 1978, 1983*b*, 1992). If there is concern about the quantity and or quality of ovarian fluid, the use of a diluent is recommended. Diluents for salmonids were defined by Nomura (1964), Billard et al. (1974), Yamamoto (1976) and Ohta et al. (1986). These solutions consist of various salts, e.g., 150 mM NaCl, and buffers, e.g., 30 mM glycine and 20 mM Tris proposed by Billard (1977*a*). Billard and Cosson (1986) and Cosson et al. (1989) found that addition of 1 mM $Ca^{++}$ prolonged sperm motility slightly. Sperm motility is inhibited when $K^{+}$ concentration is in the range of 5-80 mM (Billard and Jalabert 1974; Erdahl and Graham 1987; Billard and Cosson 1990*a*, 1990*b*) and therefore should be excluded from salmonid diluents. The improved fertilization rate and the effect of sperm concentration are both illustrated in Figure 11.

A procedure for rainbow trout fertilization that has had consistently high fertilization rates is illustrated in Figure 12. One litre of diluent (NaCl 5.52 g, glycine 3.75 g, Tris 2.42 g, mixed in 1 l of hatchery water) is added to 3 l of eggs (after ovarian fluid has been decanted). Milt (1-3 ml per litre of eggs; 1 ml milt at beginning of spawning season and 3 ml milt at end of spawning season) is then added. Mixing of eggs and sperm is done by pouring back and forth between two containers. The mixture is allowed to stand for 15 minutes. Fresh water then is added (eggs are rinsed) and the eggs then are transferred into an incubator.

There is considerable variability in sperm:egg ratios that will result in successful fertilization (i.e., > 95% fertilization success). Hence, general egg fertilization practices in salmon hatcheries empirically have been developed to have sperm:egg ratios varying between 1 to 10 million sperm per egg.

*2). Contact and rinse.* As discussed in earlier sections of this chapter, using the dry method of fertilization, sperm are activated by exposure to the ovarian fluid surrounding the eggs. Gently stirring the egg-milt mixture (without breaking eggs) and allowing a few minutes before activating the eggs (and increasing the motility of the sperm by the addition of fresh water) seems to result in optimum fertilization success (i.e., > 95 %). Hence, many eggs are fertilized by sperm prior to addition of fresh water (Ginsburg 1961). Then, the remainder are fertilized upon addition of fresh water, which causes renewed sperm motility and commences the egg-activation process. Jensen et al. (1981) varied fertilization delay (i.e., 10-40 min between egg take and fertilization), contact time (i.e., time eggs and sperm were in contact prior to water-activation; 1-10

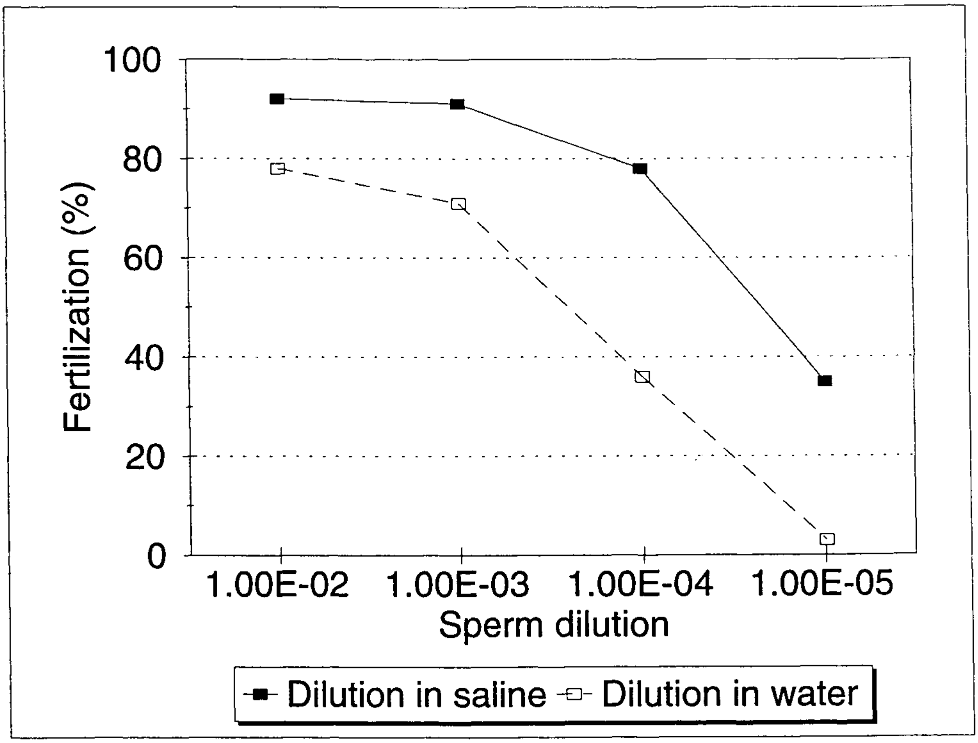

FIGURE 11. Fertilization (%) of rainbow trout eggs after insemination in saline and water at increasing dilution rates of spermatozoa ($10^{-2}$ to $10^{-5}$) (after Billard et al. 1974).

min), and the number of rinses (i.e., 1 or 2 1-min rinses with fresh water; decanting off excess sperm) on coho salmon eggs. No significant effects of any of these treatments were observed, suggesting that fish culturists have a range of options that can be employed in artificial fertilization procedures that will still yield high fertilization success. Hence, salmonid fish culturists have developed many variations in artificial fertilization. However, there are limits to these options; for example, Jensen (1975) observed that a significant ($p<0.05$) increase in coho salmon egg mortality occurred between 1 and 2 h after eggs and milt had been mixed and stored un-activated at 11°C. Salmonid culturists, while being aware of the influence of the importance of the above factors, have empirically developed many variations in artificial fertilization that yield high rates of fertilization success.

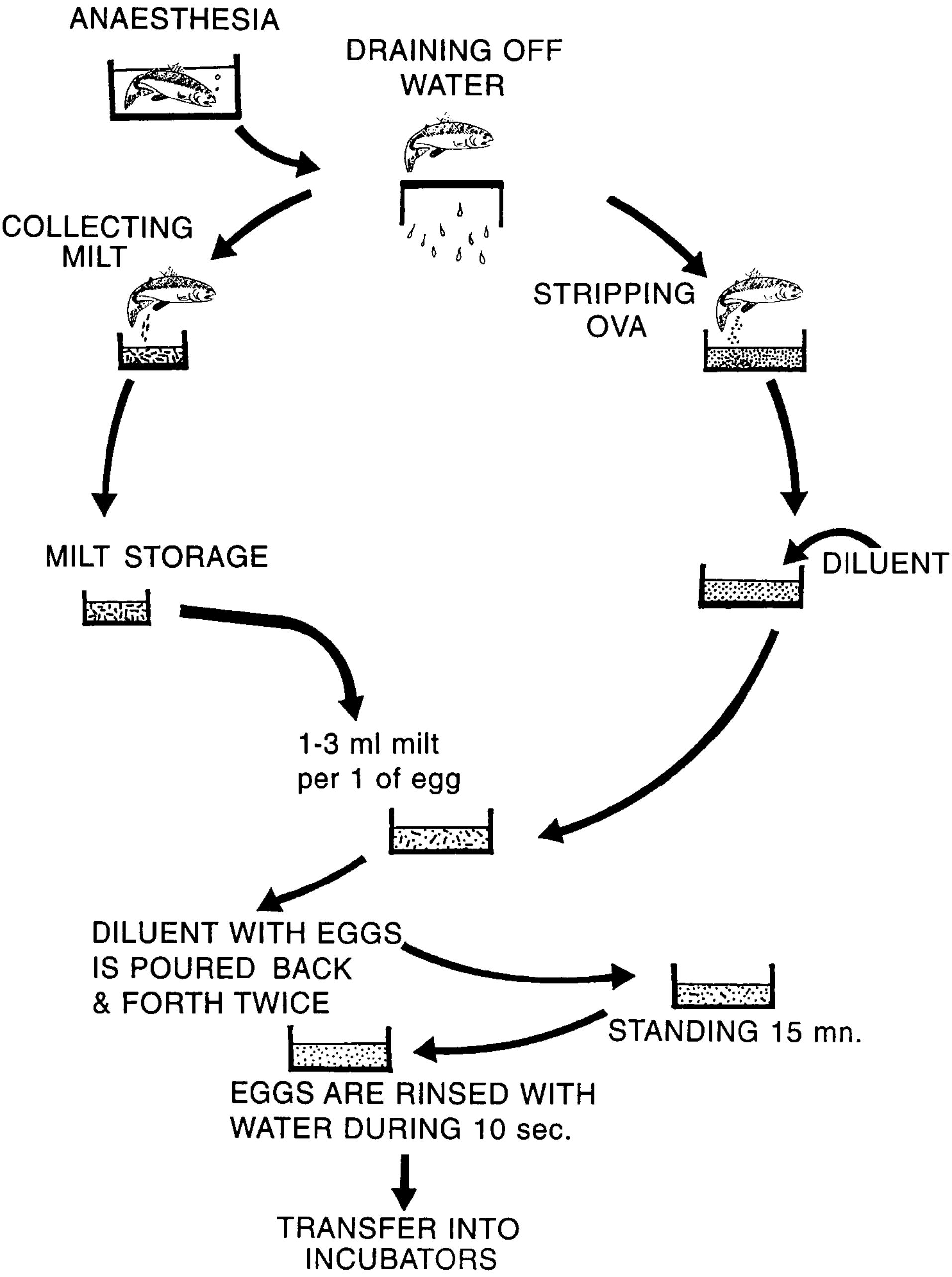

FIGURE 12. Schematic representation of the artificial fertilization procedure for salmonids using a diluent.

*Delayed fertilization.*

*1). Short-term storage of gametes (eggs and sperm).* As mentioned above, salmonid sperm are immotile in undiluted semen. To maintain optimum viability when storing milt, the requirements are low temperature, exposure to air or oxygen, sterile conditions, and prevention of desiccation (Stoss 1983; Scott and Baynes 1980). The main factor that is easily controlled is temperature. Studies of Pacific salmon gamete storage are summarized by Jensen and Alderdice (1984). The general trend of increased duration of gamete viability at low storage temperature was modelled (using an exponential decay relationship: $y = ae^{bx}$) for data compiled from sockeye (*Oncorhynchus nerka*), pink, chinook (*O. tshawytscha*), coho, and chum salmon to yield least-squares regression lines for storage times (ST, h) to 90% and 50% fertilization success in response to temperature (T, °C) as follows:

$$ST_{90\%} = 5.5582\ e^{-0.3218(T)} \qquad r = -0.890 \qquad (1)$$

$$ST_{50\%} = 6.4894\ e^{-0.3588(T)} \qquad r = -0.913 \qquad (2)$$

Gamete storage data (both eggs and sperm) were also modelled from chum salmon stored at 3, 6, 9, 12, and 15°C to yield the following two exponential relationships:

$$ST_{90\%} = 5.2808\ e^{-0.1538(T)} \qquad r = -0.980 \qquad (3)$$

$$ST_{50\%} = 5.5382\ e^{-\ 0.1325(T)} \qquad r = -0.996 \qquad (4)$$

The improved duration of gamete viability indicated by the smaller slopes (i.e., longer gamete viability) for the latter two models (Equations 3 and 4) is thought to be due to improved access to oxygen. For long-term storage (i.e., > 24 h), the addition of antibiotics and regular oxygen replenishment are recommended (Stoss 1983). More recently, McNiven et al. (1993) have successfully stored rainbow trout sperm for more than 30 days using fluoro-carbon compounds saturated with oxygen.

*2). Cryopreservation.* Cryopreservation of sperm with full retention of motility after thawing was obtained with fowl spermatozoa by addition of glycerol. Since then, extensive research has yielded great improvements in diluents and cryoprotectants that prevent cell damage during freezing and improve post-thaw fertility. Ashwood-Smith and Farrant (1980) discuss general freezing techniques. Sperm is frozen either in dry ice (−79°C) or liquid nitrogen (−196°C). Stoss (1983) discussed success of the various composition of diluents and compared sperm viability with rainbow trout sperm exposed to similar freezing and thawing techniques (Table 2, from Stoss 1983). Stoss (1983) also concluded that the numerous cryopreservation studies with spermatozoa from different salmonid species yielded results similar enough to not warrant separate discussion of species differences within this group.

TABLE 2

Fertility of rainbow trout spermatozoa frozen in various diluents[a] (modified table from Stoss 1983).

| **Diluent (sperm-to-diluent ratio 1:3)** | **DMSO concentration (%)** | **Fertility[b] (% eyed eggs) %** | **SD** | **References** |
|---|---|---|---|---|
| Distilled water | 7.5 | 67.4 | 4.0 | Stoss (1979) |
| 0.3 M glucose | 7.5 | 91.3 | 7.6 | Stoss and Refstie (1982) |
| Distilled water + 20% egg yolk | 7.0 | 82.1 | 7.9[c] | Bayrle (1980) |
| Seminal plasma | 7.5 | 6.4 | 2.3 | Bayrle (1980) |
| Hfx#10[d] | 7.5 | 83.3 | 4.8 | Truscott and Idler (1969) |
| Diluent[e] | 7.5 | 93.9 | 3.7 | Mounib (1978) |
| Diluent[f] | 7.5 | 87.7 | 4.1 | Stoss and Holtz (1983*a*) |
| Diluent[g] | 7.5 | 88.2 | 2.9 | Stein and Bayrle (1978) |

[a] Pellet technique, thawing in 120 mmole $NaHCO_3$ (10°C)
[b] Fresh sperm control is 100%.
[c] Sperm dilution after thawing 1/115.
[d] Media contains 103 mM NaCl, 22mM KCl, 1.3 mM $CaCl_2$, 0.5 mM $MgSO_4$, 3.3 mM fructose, 79.9 mM glycine.
[e] Media contains 125 mM sucrose, 6.5 mM glutathione, 100 mM $KHCO_3$.
[f] Media contains 101 mM NaCl, 23 mM KCl, 5.4 mM CaCl, 1.3 mM $MgSO_4$, 200 mM Tris-citric acid (pH 7.25), 0.4% bovine serum albumin, 0.75% Promine D.
[g] Media contains 128.3 mM NaCl, 23.8 mM $NaHCO_3$, 3.7 mM $Na_2HPO_8$, 0.4 mM $MgSO_4$, 5.1 mM KCl, 2.3 mM CaC12, 5.6 mM glucose, 66.6 mM glycine, 20% egg yolk.

Scott and Baynes (1980) reported that much progress had been made since the subject was reviewed by Horton and Ott (1976), with levels of fertilization success of up to 90% reported. Scott and Baynes (1980), in reviewing the published information on sperm cryopreservation, discussed factors that influenced the development of successful cryopreservation techniques. They indicated that initial temperature at the time of sperm collection and prior to cryopreservation was important and that milt, all containers, and diluents and extenders should be kept cool (refrigerated) prior to use, and that diluted milt should be kept refrigerated until commencement of cryopreservation. For storage temperature they recommended the use of liquid nitrogen (−196°C) as the most practical for long-term storage. Mounib (1978) reported Atlantic salmon sperm viability of 80% after 1 yr of storage in liquid nitrogen at −196°C, with much longer storage periods expected (Scott and Baynes 1980). Storage techniques vary from the use of capped glass or polypropylene vials (2 ml volume), pellets of diluted milt formed by freezing samples in indentations in dry ice, or the use of straws, sealed at both ends, and frozen above the liquid nitrogen level. Reported volumes of samples ranged from 40-$\mu$l pellets to 1000-$\mu$l vial samples. Milt cannot be successfully cryopreserved unless diluted in an extender. Scott and Baynes (1980) discussed the important components of extenders, including NaCl, KCl, $Mg^{++}$, $Ca^{++}$, buffers, sugars, glycine, mannitol, glutathione, and phospholipids, lipoproteins and proteins. Dilution ratios used are 1 part milt:3-4 parts extender. Rate of freezing, the necessity of a cryoprotectant such as dimethyl sulphoxide (DMSO), and subsequent rate of thawing were also discussed by Scott and Baynes (1980). For a detailed summary of these factors, readers are referred to Scott and Baynes (1980) and Legendre and Billard (1980).

Fish sperm cryopreservation has recently been shown to be related to the biochemical composition of the plasma membrane. It is known that the sperm of marine fish species is easier to freeze than sperm of freshwater species. Drokin (1993) suggested this may be due to the high cholesterol:phospholipid ratio of plasma membrane of marine fish spermatozoa. Malejac et al. (1990) and Maisse et al. (1988) also related the success of freezing to the quality of the sperm membrane.

Of the cryoprotectants tested for salmonid sperm, DMSO provided good protection at concentrations of 6.8-12.5% (v/v, final concentration) (Stoss and Holtz 1983*b*). Glycerol has a deleterious effect on unfrozen sperm. Ethylene glycol did not protect brown trout sperm (Stein 1979, in Stoss 1983). Propylene glycol was as effective as DMSO in protecting Atlantic salmon sperm (Truscott and Idler 1969). Polyvinylpyrrolidone did not protect coho salmon nor brown trout sperm (Ott and Horton 1971; Stein 1979, in Stoss 1983). Stoss (1983) suggested that the rates of freezing and thawing are the most critical factors affecting sperm viability when cryopreserved. Morphologically damaged sperm have been observed when frozen sperm are thawed (Billard and Breton 1970; Billard 1983*c*) and Lahnsteiner et al. (1992) reported 10-20% intact thawed sperm in grayling (*Thymallus thymallus*).

*Genetic Considerations and Fertilization Scheme.* Over the years, many populations of trout have been domesticated and desirable traits, such as timing of maturation, egg survival and growth rate have been selected. Hence, the resultant genetic composition of cultured salmonids has been of concern. The undesirable effects of inbreeding can be minimized by maintaining a large number of broodstock (Hynes et al. 1981; Krueger et al. 1981; *see* Chapters 4 and 11). Furthermore, using equal numbers of male and female parents will help to ensure maximum genetic diversity by contributing equally to the next generation (Falconer 1960; Allendorf and Phelps 1980; Gharrett and Shirley 1985).

In Pacific salmon hatcheries, it was common practice to, until recently, pool the milt from several males and apply this milt to one or more batches (one batch of eggs coming from one female) of eggs before water was added. It was assumed that each male fertilized an equal number of eggs, proportionally to the milt volume and sperm concentration. Withler (1988) showed that this was not the case. As mentioned above, sperm are activated in ovarian fluid and penetrate the egg prior to egg-activation by fresh water. Using electrophoretic data to identify the paternity of progeny resulting from individual and pooled crosses with 10 males and 4 females, Withler (1988) compared observed and expected progeny types. With pooled milt, individual male fertilization varied between 1.4 to 75.6%, with significant heterogeneity between replicate crosses. In addition, potency of individual males was not correlated with spermatocrit, and varied between mixtures of pooled milt. Withler (1988) concluded that pooling milt could reduce the number of males that fertilize eggs and hence increase the variance in family size that contributes to fertilization, resulting in an accelerated loss of genetic diversity. To avoid this problem (particularly with broodstocks of fewer than 200 fish), Withler (1988) recommended that milt from an individual male be used to fertilize eggs from an individual female.

## INCUBATION

### *Egg Development*

*Water-hardening.* The water-hardening process (i.e., cortical vesicle exocytosis, imbibition of water and egg capsule changes) occurs when eggs enter fresh water. Cortical granules are released as part of the egg activation process. These large molecules create an osmotic gradient causing the rapid uptake of water through the semipermeable egg capsule. A perivitelline space between the egg capsule and the vitelline membrane covering the yolk is formed (99% of water imbibition is complete after 1 h) and becomes filled with perivitelline fluid, increasing the total volume of the egg by about 20%. Over the next several days, the egg capsule has been shown to "harden" (a process not well understood) and increase in elastic strength (Zotin 1958). These changes are felt to be important to protect the developing embryo from mechanical shock. The rates of water uptake and egg capsule strength have been shown to be adversely affected by low pH (Rombough and Jensen 1985).

*Development Rates.* Teleost eggs, being poikilotherms, are strongly influenced by temperature during embryonic development. The accumulated temperature units (ATUs) to a specific developmental stage is often considered to be a constant for a species:

$$ATU = T\,D = k = \text{constant} \tag{5}$$

where D = number of days to a particular stage, T = average temperature to that stage, and k = constant for a species. The time to hatch for any temperature regime can then be predicted from k/T. This model is reasonably accurate at temperatures greater than 5°C but increasingly overestimates incubation times at lower temperatures. To overcome this difficulty many models have been compared (Alderdice and Velsen 1978; Crisp 1981, 1988; Jungwirth and Winkler 1984; Elliott et al. 1987; Beacham and Murray 1990). McLean et al. (1991) used two models to describe egg and larval development rates using data compiled by Velsen (1987), Murray and McPhail (1988), and Rombough (unpubl. data). The first model, often called the Bělehrádek model (Bělehrádek 1930), is described by the equation:

$$D = a\,/\,(T - c)^{b} \tag{6}$$

which rearranges to yield ATU as follows:

$$ATU = D\,T = T\,(a\,/\,(T - c)^{b}) \tag{7}$$

where a, b, and c are constants characteristic of a species and stage of development. This model was used to predict time-temperature relations for two embryonic stages (yolk plug closure and eyed stages) described by Ballard (1968) using the data from Velsen (1987).

A second model, originally proposed by Schnute (1981) to describe growth curves for fish, was found to give a better fit to the time-temperature data for salmonid egg and larval development. However, this model is more complex and requires larger data sets than the Bělehrádek model. Therefore, it was only used to model time to hatch, for which the data sets were sufficiently large. The model, with constants a and b not equal to zero, is described by the equation:

$$D = \frac{[D_1^{\,b} + (D_2^{\,b} - D_1^{\,b})(1 - e^{-a(T - T1)})]^{1/b}}{(1 - e^{-a(T2 - T1)})} \tag{8}$$

where $T_1$ and $T_2$ are the minimum and maximum incubation temperatures (°C) from the data set used, and $D_1$ and $D_2$ are the predicted development times (days) at those temperatures. Calculation of ATUs was then simply made by multiplying the predicted number of days to hatch (D) and the average temperature (T). The model coefficients are listed in Table 3 and a series of predicted times (days and ATUs) to three development stages (yolk plug closure, eyed, and 50% hatch) for five Pacific salmon species and steelhead trout at 5, 7.5, 10, and 12.5°C were generated (Table 4) using the models listed in Table 3.

TABLE 3

Equation coefficients[1] for three development stages (completion of epiboly, eyed, and hatch) modelled for each of the six species and the number of data points (N) (from McLean et al. 1991).

| Species and stage | a | b | c | D1 | D2 | N |
|---|---|---|---|---|---|---|
| Chinook | | | | | | |
| epiboly | 207.1222814 | 1.143230343 | −1.00175611 | | | 7 |
| eyed | 883.2385275 | 1.414574147 | −2.45746387 | | | 7 |
| hatch | 0.098319 | −0.0371603 | | 239.1 | 27.8 | 295 |
| Chum | | | | | | |
| epiboly | 510.6446646 | 1.503134190 | −1.32571468 | | | 5 |
| eyed | 2378.325397 | 1.761904580 | −3.94778141 | | | 5 |
| hatch | 0.1362313 | 0.7193096 | | 177.8 | 25.8 | 101 |
| Coho | | | | | | |
| epiboly | 13030348.207 | 3.436728916 | −19.2127868 | | | 5 |
| eyed | 16661.25802 | 2.255270212 | −8.61837921 | | | 5 |
| hatch | 0.0964044 | 0.0790329 | | 213.2 | 21.6 | 108 |
| Pink | | | | | | |
| epiboly | 704.6378875 | 1.567007981 | −1.59306900 | | | 6 |
| eyed | 390.088759 | 1.209049777 | −0.34303429 | | | 6 |
| hatch | 0.5672965 | 4.4479176 | | 180.4 | 49.7 | 54 |
| Sockeye | | | | | | |
| epiboly | 1071672210 | 5.109288900 | −21.5645854 | | | 3 |
| eyed | 1097342643 | 4.801222359 | −29.0742377 | | | 3 |
| hatch | 0.1730653 | 1.0483823 | | 212.4 | 36.4 | 81 |
| Steelhead | | | | | | |
| epiboly | 394.1655832 | 1.500516000 | −2.94168479 | | | 4 |
| eyed | 3223106353 | 5.173971576 | −29.7568559 | | | 4 |
| hatch | 0.4084140 | 2.3613821 | | 139.3 | 18.3 | 127 |

[1] Equation (7) was used to describe time to completion of epiboly and the eyed stage. Equation (8) was used to describe time to hatching, with auxiliary parameters for all species set at $T_1$ = 1°C and $T_2$ = 20°C

For fluctuating non-constant temperatures, another approach to development rate prediction has been to model daily development rate (% development/day) by the following data conversion:

$$P(\%\ \text{development/day}) = 100/y \qquad (9)$$

where y is the number of days to 50% hatch at a given temperature. Alderdice and Velsen (1978) used this approach in modelling chinook salmon egg development to predict time to 50% hatch for non-constant temperature regimes.

For other species of salmonids, several authors (Crisp 1981, 1988; Jungwirth and Winkler 1984; Elliott et al. 1987) have developed models to predict development time to some embryonic stages, hatching and emergence. The species include Arctic charr (*Salvelinus alpinus*), Atlantic salmon, brook trout, brown trout, cutthroat trout, Danube salmon or huchen (*Hucho hucho*), and rainbow trout. Model coefficients from these empirical mathematical models for predicting time to hatching were compiled into Table 5 and used to create Table 6, similar to Table 4 for Pacific salmon species and steelhead trout.

### *Sensitivity to Mechanical Shock and Implications for Culture Protocols*

Salmonid eggs generally exhibit significant sensitivity to handling or movement, commonly referred to as mechanical shock sensitivity, from fertilization to the eyed stage. Different methods have been used to quantify the changes in sensitivity that occur during this early phase of egg development. These approaches have included pouring groups of eggs from various heights (Battle 1944; Smirnov 1954; Jensen et al. 1981), dropping eggs in simulated gravel beds (Post et al. 1974), simulating water current disturbances over a redd with jet boats (Sutherland and Ogle 1975), and vibrating eggs on incubation screens (Hata 1927; Smirnov 1954, 1955, 1975; Ielava 1967). From the earlier studies that generally were not quantifiable in terms of shock applied, it appeared that eggs could be gently handled during the first few minutes following egg-activation. Sensitivity was then thought to increase rapidly to a peak about 15 min post-activation. Eggs were then left undisturbed for 1.5-3 h and allowed to water-harden. After this period, eggs were assumed to be less sensitive to shock and could be safely moved, transported and placed in incubators. Eggs were then thought to become highly sensitive again, 8-10 h after activation, remaining sensitive until the eyed stage.

Jensen and Alderdice (1983, 1989) used a device to expose salmonid eggs to standardized, quantifiable shock intensities. The mechanical shock apparatus, consisted of a carrier with a slot to hold a 60-mm diameter × 15-mm plastic petri dish containing a single layer of about 30 salmonid eggs. The carrier could initially be raised from 0-50 cm (later increased to 100 cm), from where the carrier was released, falling freely (guided by two metal wires). Shock tests were performed on six salmonid species (using eggs from the unactivated stage to commencement of hatching). At various time intervals, eggs were exposed to a series of shocks to obtain estimates of 50% and 10% mortality

TABLE 4

Predicted embryonic development times (in days and ATUs) for five Pacific salmon species and steelhead trout (using the models listed in Table 3).

| **Species** | **Temp. °C** | **Yolk plug closure** | | **Eyed stage** | | **50% hatch** | |
|---|---|---|---|---|---|---|---|
| | | **Days** | **ATUs** | **Days** | **ATUs** | **Days** | **ATUs** |
| Chinook | 5 | 26.7 | 133.5 | 51.5 | 257.5 | 102.4 | 511.8 |
| | 7.5 | 17.9 | 134.5 | 34.2 | 256.6 | 70.3 | 527.5 |
| | 10 | 13.4 | 133.5 | 24.9 | 249.2 | 52.6 | 526.4 |
| | 12.5 | 10.6 | 132.1 | 19.2 | 240.5 | 42.1 | 525.7 |
| Chum | 5 | 31.9 | 159.6 | 50.1 | 250.3 | 99.6 | 498.2 |
| | 7.5 | 19.3 | 145.1 | 32.4 | 243.3 | 72.3 | 542.3 |
| | 10 | 13.3 | 133.0 | 22.9 | 229.0 | 54.4 | 544.5 |
| | 12.5 | 9.9 | 123.2 | 17.1 | 214.1 | 42.7 | 533.2 |
| Coho | 5 | 22.8 | 114.1 | 46.1 | 230.6 | 93.6 | 467.8 |
| | 7.5 | 16.3 | 122.1 | 31.5 | 236.6 | 63.1 | 473.6 |
| | 10 | 12.0 | 119.7 | 22.8 | 227.8 | 45.9 | 459.5 |
| | 12.5 | 9.0 | 112.9 | 17.1 | 214.4 | 35.6 | 444.8 |
| Pink | 5 | 36.7 | 183.4 | 51.4 | 257.2 | 109.0 | 545.0 |
| | 7.5 | 22.2 | 166.2 | 32.3 | 242.5 | 80.9 | 606.4 |
| | 10 | 15.1 | 151.5 | 23.1 | 231.4 | 63.0 | 629.6 |
| | 12.5 | 11.2 | 139.4 | 17.8 | 222.7 | 54.0 | 674.9 |
| Sockeye | 5 | 27.3 | 136.4 | 48.2 | 240.9 | 122.8 | 613.8 |
| | 7.5 | 18.3 | 137.0 | 34.3 | 257.2 | 90.5 | 679.0 |
| | 10 | 12.6 | 126.0 | 25.0 | 249.6 | 69.3 | 693.2 |
| | 12.5 | 8.9 | 111.4 | 18.5 | 231.7 | 55.4 | 692.5 |
| Steelhead | 5 | 17.6 | 88.0 | 34.3 | 171.4 | 70.7 | 353.4 |
| | 7.5 | 11.7 | 87.5 | 23.9 | 179.5 | 47.2 | 354.0 |
| | 10 | 8.5 | 84.6 | 17.1 | 171.0 | 32.9 | 328.6 |
| | 12.5 | 6.5 | 81.1 | 12.5 | 155.9 | 24.8 | 309.8 |

**TABLE 5**

Equation coefficients for time to hatch, modelled for seven salmonid species using three models (i.e., curvilinear or log-transformed Bělehrádek model [Equation 6], Schnute growth model [Equation 8], while a variation of the Power Law was used to model three data points for cutthroat trout). The number of equations (i.e., different sources of data) for each species varied from 1 to 3.

| Species | Model coefficients | | | | | | | Model | Source |
|---|---|---|---|---|---|---|---|---|---|
| | a | log a | b(1) | b2 | c | D1 | D2 | | |
| Arctic charr | 476.76 | | 0.1314 | | 1.0435 | | | $y=a/(x-b)^c$ | Jungwirth and Winkler (1984) |
| | 196.2 | | 0.14 | 0.0042 | | | | $a^*\exp(-b1T-b2T^2)$ | Elliott et al. (1987) |
| Atlantic salmon | | 5.1908 | −2.6562 | | −11 | | | $\log D=b\log(T-c)+\log a$ | Crisp (1981) |
| Brook trout | | 19.6765 | −9.5948 | | −65 | | | $\log D=b\log(T-c)+\log a$ | Crisp (1981) |
| | 32437 | | −8.73 | | 2.25 | | | $y=a/(x-b)^c$ | Elliott et al. (1987) |
| Brown trout | | 28.8392 | −13.9306 | | −80 | | | $\log D=b\log(T-c)+\log a$ | Crisp (1981) |

Table 5 cont'd.

| Species | Model coefficients | | | | | | | Model | Source |
|---|---|---|---|---|---|---|---|---|---|
| | a | log a | b(1) | b2 | c | D1 | D2 | | |
| Brown trout cont'd. | 71767.3 | | −8.49 | | 2.58 | | | $y=a/(x-b)^c$ | Elliott et al. (1987) |
| | 746 | | −0.5323 | | 1.2233 | | | $y=a/(x-b)^c$ | Jungwirth and Winkler (1984) |
| Cutthroat trout | −60.76908 | | 291.9083 | | | | | $y=a+b/x^{0.5}$ | Merriman (1935) |
| Danube salmon | 98 | | −3.12 | | 0.78 | | | $y=a/(x-b)^c$ | Elliott et al. (1987) |
| | 2647.4 | | −3.22 | | 1.7865 | | | $y=a/(x-b)^c$ | Jungwirth and Winkler (1984) |
| Rainbow trout | | 4.0313 | −2.0961 | | −6 | | | $\log D=b \log(T-c)+\log a$ | Crisp (1981) |
| | 979.4 | | −1.53 | | 1.37 | | | $y=a/(x-b)^c$ | Elliott et al. (1987) |
| | 0.4084140 | | 2.3613821 | | | 139.3 | 18.3 | see Equation 8[a] | McLean et al. (1991) |

[a] Equation (8) was used to describe time to hatching, with auxiliary parameters set at $T_1$ = 1°C and $T_2$ = 20°C.

**TABLE 6**

Predicted embryonic development times (in days and ATUs) to hatch for various salmonid species based on models listed in Table 5 (i.e., time to hatch was determined by taking the average of up to three modeled predictions for temperatures ranging from 5 to 12.5°C).

| Species | Temperature | Time to 50% hatch | |
|---|---|---|---|
| | °C | days | ATUs |
| Arctic charr | 5 | 89.6 | 447.8 |
| | 7.5 | 56.8 | 425.7 |
| | 10 | 37.8 | 377.6 |
| | 12.5 | 26.1 | 326.5 |
| Atlantic salmon | 5 | 98.3 | 491.3 |
| | 7.5 | 66.8 | 501.2 |
| | 10 | 47.7 | 477.2 |
| | 12.5 | 35.4 | 442.5 |
| Brook trout | 5 | 91.7 | 458.5 |
| | 7.5 | 64.2 | 481.8 |
| | 10 | 46.5 | 464.7 |
| | 12.5 | 34.5 | 430.8 |
| Brown trout | 5 | 90.4 | 452.0 |
| | 7.5 | 57.9 | 434.0 |
| | 10 | 38.3 | 383.0 |
| | 12.5 | 26.0 | 325.5 |
| Danube salmon (huchen) | 5 | 60.7 | 303.3 |
| | 7.5 | 34.6 | 259.5 |
| | 10 | 24.0 | 240.3 |
| | 12.5 | 18.2 | 227.4 |
| Rainbow trout | 5 | 87.2 | 436.0 |
| | 7.5 | 56.2 | 421.8 |
| | 10 | 38.7 | 386.6 |
| | 12.5 | 27.9 | 348.4 |

($LD_{50}$ and $LD_{10}$, respectively), expressed either as drop height (cm) or in terms of energy imparted (ergs, g $cm^2$ $s^{-2}$). All tests were conducted at 10°C. Therefore, since teleost development is temperature-dependent, changes in shock sensitivity were reported in terms of stages of development (until the detection limit of the shock test was reached, between stage 19 [yolk surface 2/3 vascularized] and stage 20 [eyes fully pigmented]) as well as time (Jensen and Alderdice 1989).

From these tests, a general pattern emerged. Surprisingly, all eggs exhibited significant sensitivity to shock prior to activation with estimates of drop heights causing 10% mortality ranging from 12.1-41.7 cm (Jensen and Alderdice 1989). Hence, fish culturists should minimize the drop-height when taking eggs. After fertilization, egg sensitivity followed a log-linear increase until about stage 8 (spreading into a flattened blastodisc). This was followed by a parabolic-shaped response, with the maximum sensitivity occurring at stage 10-11 (⅓ to ½ epiboly), with drop heights causing 50% mortality ranging from 2.83-11.28 cm (Figure 13).

To avoid egg loss due to mechanical shock, the following are recommended:

(1) Normal manipulation of eggs (insertion into trays, boxes) should be completed within 10 min following activation;
(2) Transport eggs unfertilized if possible;
(3) If eggs must be transported following activation, then this should be done with eggs closely packed to prevent movement, and this should be done soon after activation, as sensitivity continues to increase from fertilization until stage 10 -11; and
(4) Eggs should not be moved until completion of epiboly (yolk plug closed; stage 14), and then only gently until the embryos' eyes are fully pigmented (stage 20), at which time eggs are very resistant to mechanical shock.

### *Types of Incubators and Their Uses*

Numerous shapes and sizes of incubators have been designed and used successfully by salmonid culturists. The sizes and shapes vary among trays, troughs, and boxes. The basic requirement is a container with sufficient water flowing through it and some type of screened enclosure to prevent eggs or larvae from being washed away. Also, some species of salmonids require a substrate for larval development to proceed normally. Specific features have prompted certain requirements.

*Vertical-flow Stack Incubator.* The common vertical-flow stack of incubation trays, often called a Heath stack, consists of a stack of 8 to 16 trays with water flowing into the back of each tray, then flowing up through a screened insert-tray, and finally overflowing into the tray below. The advantage of such a system is that eggs from individual females can be kept separate, allowing fish culturists to easily monitor egg development and survival in relation to individual traits or specific treatments that single fish may have

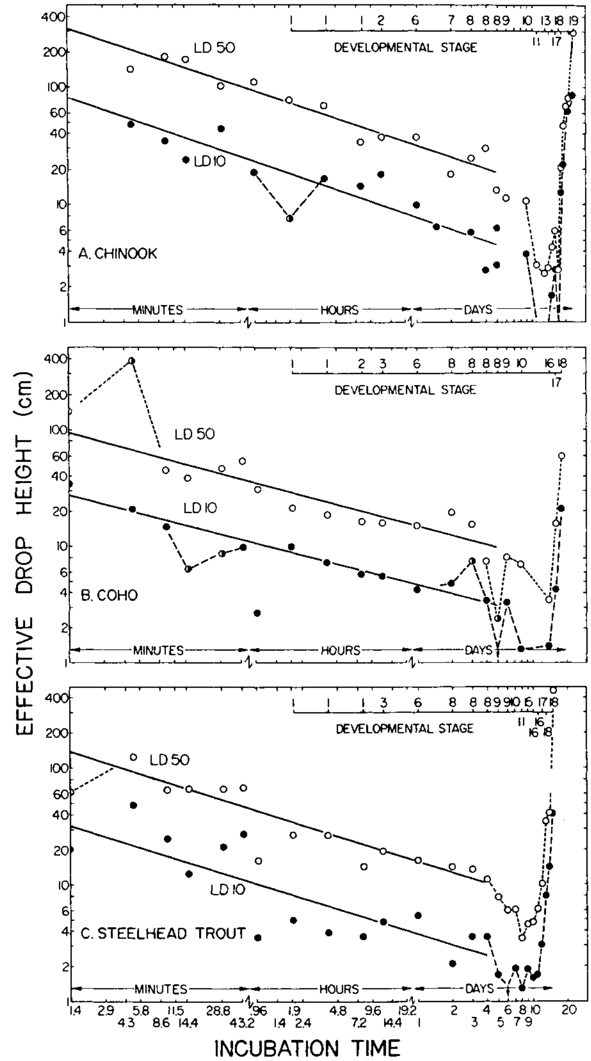

FIGURE 13. Shock sensitivities (LD50, LD10; cm) in five species of Pacific salmon and steelhead trout (Panels A to F) from fertilization to stages 1 - 19 (*see* Table 11) at 10°C. The distributions are in two sections: (A) a log-linear section from fertilization to stage 8; and on the following page, (B) a parabolic section from stage 9 to about stage 19. Development stages are shown in the upper right corner of each panel. Both linear and parabolic sections were fitted after removal of outliers (shown as half-solid points). Beyond embryonic stages 17 - 19, the eggs beacme relatively insensitive to mechancial shock. Representative confidence intervals (95%) are shown in Panel F (sockeye) for the LD50 distribution (from Jensen and Alderdice 1989).

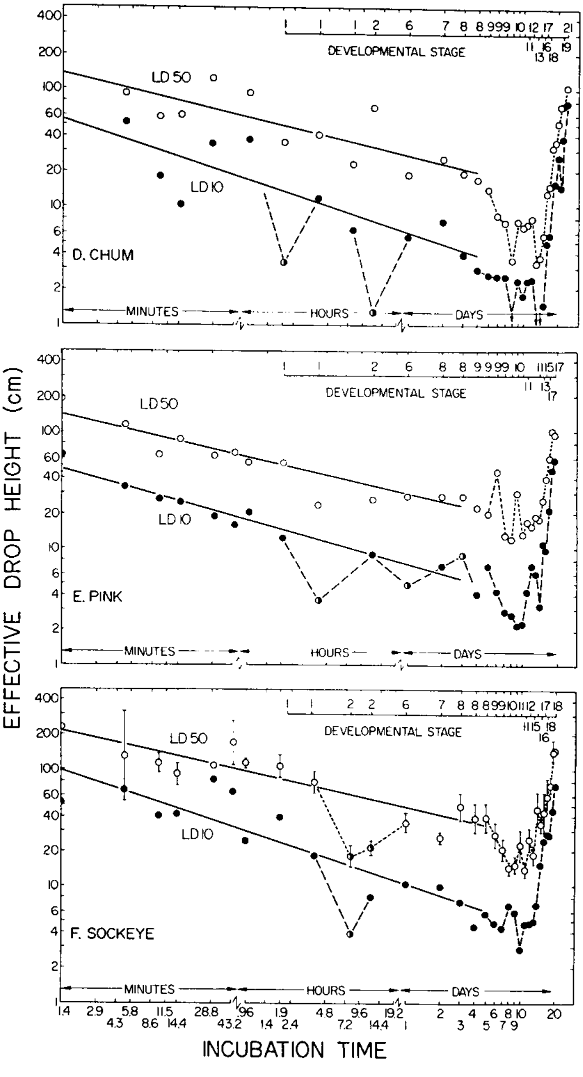
D. CHUM
E. PINK
F. SOCKEYE
LD 50
LD 10
DEVELOPMENTAL STAGE
MINUTES
HOURS
DAYS
EFFECTIVE DROP HEIGHT (cm)
INCUBATION TIME
400
200
100
60
40
20
10
6
4
2
1
1.4 2.9 5.8 11.5 28.8 .96 1.9 4.8 9.6 19.2 2 4 6 8 10 20
4.3 8.6 14.4 43.2 1.4 2.4 7.2 14.4 1 3 5 7 9

FIGURE 13(B).

been exposed to. Also, it is simple to discard a single bad batch (eggs of poor quality) when eggs from individual females are kept separate. It is generally easier to handle, monitor and observe egg development and hatching success in the relatively small groups of eggs held in these types of incubators (Figure 14).

*Troughs.* The main principle of incubating eggs in troughs is to introduce water at one end of the trough and direct the flow through a mass of eggs. This is achieved by having a series of baffles that push water down and then up through the eggs (which are held either in baskets or are placed on perforated screens). Leitritz and Lewis (1976) describe the use of baskets in troughs. Trough incubators typically are used for incubating large numbers of mixed groups of eggs (ranging from 20,000 to 100,000 eggs per basket or trough-cell). The advantage of this type of incubator is that large groups of eggs can quickly be handled and incubated (Figure 15).

*Boxes.* Large box-like containers with upwelling water flow (Figure 16) also have been used for incubating even larger numbers of mixed groups of eggs (500,000 or more) in a single container. Such incubators further reduce the time required in fertilizing and handling the eggs as well as reducing overall space requirements for an incubation room. This approach to egg incubation works well as long as all eggs are of good quality and high fertilization is achieved.

*Substrate.* Several investigators have found that substrate (consisting of either gravel, various-shaped plastic, or even artificial turf) has improved survival and growth of certain salmonid species. These species include chum salmon (Murray and Beacham 1986), pink salmon (Bams 1972) and Atlantic salmon (Hansen and Møller 1985; Hansen and Torrissen 1985). Also, Emadi (1973) observed yolk-sac malformation in chum, pink, sockeye, and hybrid (pink × chinook) salmon alevins in smooth-surface incubators, but no yolk-sac malformation in chinook and coho alevins. The substrate becomes important during the larval period from hatch to complete yolk absorption, helping to support larvae, prevent damage to yolk-sacs and reduce excessive swimming activity (Figure 16).

### *Review of Critical Water Quality Requirements During Incubation*

The primary water quality requirements for salmonid eggs and larvae are temperature, dissolved gases (oxygen and nitrogen), pH, water hardness or mineral content, and metabolic waste products (ammonia and carbon dioxide). The carrying capacity (i.e., the maximum number of eggs that can be safely incubated per unit of water) of a hatchery water supply is dependent on the constraints imposed by the effects of these factors on the health and survival of developing salmonid eggs.

*Temperature.* For Pacific salmon and steelhead trout, minimum and maximum temperature limits were proposed (Sigma Resources 1983), based on upper and lower incipient lethal levels, and are presented in Table 7. These estimates are based on

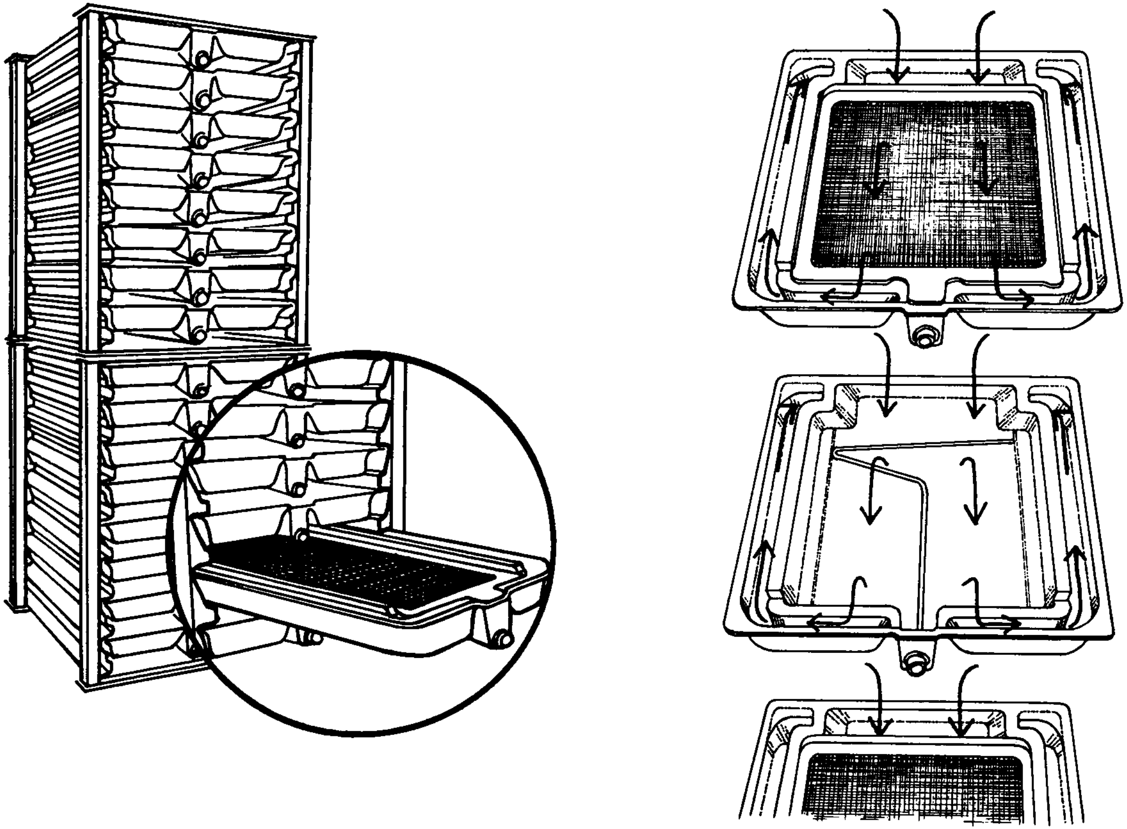

FIGURE 14. An example of a 16-tray vertical incubator configuration (left). Illustration of three trays (right), showing the direction of water flow (indicated by arrows) through each tray and into the next.

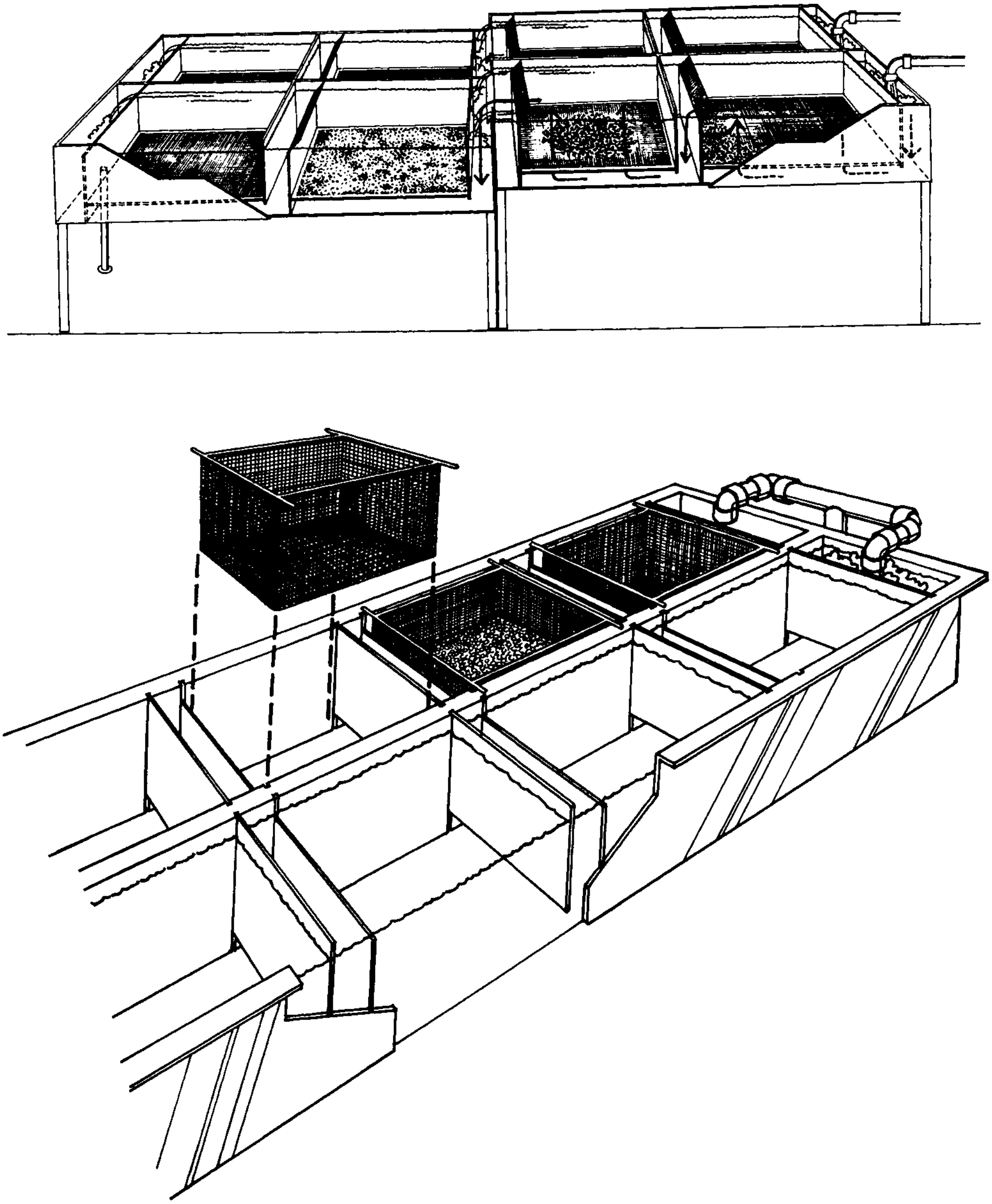

FIGURE 15. Illustrations of two types of troughs. The first type (top) uses a staggered configuration of two large sections. The second type (bottom) is a straight trough with baffles that are removed for initial feeding.

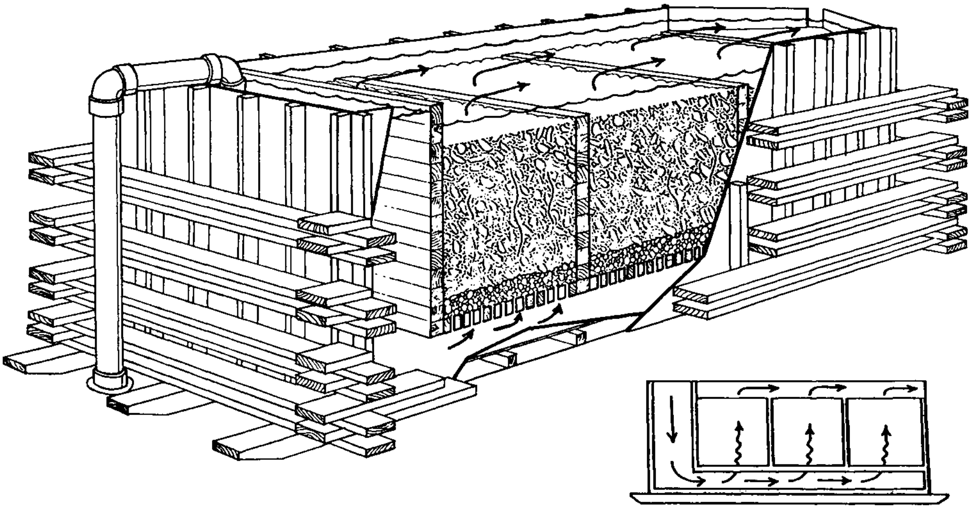

FIGURE 16. Illustration of a gravel box-incubator. The small side view shows the typical water flow pattern that ensures good exchange through the gravel substrate.

experimental data obtained when eggs were incubated at constant temperatures from fertilization to hatching. Peterson et al. (1977) exposed Atlantic salmon eggs and larvae to systematically varied temperatures ranging from 2 to 12°C for three developmental phases (i.e., from fertilization to the eyed stage, from eyeing to 50% hatch, and after hatching). Exposure to <4°C immediately after fertilization caused more than 20% egg mortality, while temperatures >4°C resulted in 5% mortality or less. Sudden decreases in temperature at the eyed-egg stage and at hatching caused edema in the larval yolk sac, resulting in mortality and decreased growth. From Velsen (1987) and Beacham and Murray (1990) it appears that the order of low temperature tolerance for Pacific salmon (most tolerant to least tolerant) is coho, sockeye, chinook, chum, and pink salmon; while the order of high temperature tolerance is chinook, chum, pink, sockeye, and coho. Genetic adaption to local differences in temperature has also been noted; for example, Beacham and Murray (1990) point out that chum salmon eggs in the Yukon river develop faster (per temperature unit) than comparable populations in southern British Columbia.

*Dissolved Oxygen.* Dissolved oxygen (DO) is essential for all animal life and is the main limiting factor in determining the carrying or loading capacity of an incubation environment (both natural spawning redds or artificial incubators). A reduction in DO will slow embryonic development and result in reduced growth rate and yolk conversion efficiency. If DO levels are extremely low, abnormal and deformed embryos (Alderdice

TABLE 7

Minimum and maximum temperature limits for incubation based on constant temperatures (from Sigma Resources 1983).

| Species | | Minimum temperature °C | | Maximum temperature °C | |
|---|---|---|---|---|---|
| | | ILL[1] | Safe[2] | ILL[1] | Safe[2] |
| chinook | | 2.75 | 4.5 | 15.8 | 14 |
| chum | <[3] | 3.7 | 5.0 | 15.8 | 14 |
| coho | <[3] | 1.6 | 3.0 | 13.0 | 10 |
| pink | | 3.8 | 5.0 | 15.6 | 12 |
| sockeye | | 2.7 | 5.0 | 17.5 | 12 |
| steelhead[4] | <[3] | 2.25 | 3.0 | 14.6 | 11 |

[1] Incipient lethal level, 50% mortality, from fertilization to hatch.

[2] Suggested level that will not cause significant mortality, yet may not be optimal for growth and health.

[3] Incipient lethal level less than reported; data insufficient to define ILL.

[4] Both sea-run steelhead and non-migratory rainbow trout are included in this table.

et al. 1958) or death will occur. As long as DO levels around salmonid eggs remain sufficiently high, embryos will respire and develop normally. However, there exists a threshold or incipient level called the critical DO level (Pc), that can be determined experimentally, below which reduced metabolism and growth rates occur.

Rombough (1986) and McLean et al. (1991) developed oxygen consumption (Ro) and Pc models for steelhead and five Pacific salmon species for eggs from fertilization to hatch and larvae to fry emergence (*see* Tables 8 and 9, from McLean et al. 1991). These models predict Ro and Pc in response to temperature and stage of development as determined by the development models (Table 3) and accumulated temperature units (ATUs). Hence, salmonid fish culturists can use these models to calculate the changing Ro levels with time and temperature and ensure that developing eggs have adequate water flow to maintain DO above Pc levels. To make these calculations easier, the models described in McLean et al. (1991) were included in a computer program (SIRP) described below (Jensen et al. 1992).

*Total Gas Pressure.* Total gas pressure (TGP) is the sum of partial pressures of all dissolved gases in a solution. The two most abundant gases are oxygen ($O_2$) and nitrogen ($N_2$), comprising about 21 and 79% of our atmosphere (with all other gases

TABLE 8

Oxygen consumption rates (Ro; mg $O_2$ /1000 eggs · $h^{-1}$) for five Pacific salmon species and steelhead trout as a function of accumulated temperature units (ATUs; °C days) and ambient temperature (T; °C) for two developmental phases: (1) fertilization to completion of epiboly and (2) completion of epiboly to MAWW or fry emergence. The $R^2\%$ (where sufficient data exist) and the N (number of data points) also are shown (from McLean et al. 1991).

| Phase | Equation |
|---|---|
| | Chinook |
| 1. | $\ln(Ro) = -2.780 + 0.944\ln(T)$; $R^2 = 99.8$, N = 5 |
| 2. | $\ln(Ro) = -16.897 + 2.873\ln(ATU/T) + 3.840\ln(T)$; $R^2 = 96.3$, N = 75 |
| | Coho |
| 1. | $Ro = -0.240 + 0.364\ln(T)$; $R^2 = 90.2$, N = 5 |
| 2. | $\ln(Ro) = -15.417 + 2.746\ln(ATU/T) + 3.528\ln(T)$; $R^2 = 96.3$, N = 74 |
| | Chum |
| 1. | $Ro = 0.60$; N = 2 |
| 2. | $\ln(Ro) = -15.437 + 2.774\ln(ATU/T) + 3.212\ln(T)$; $R^2 = 95.7$, N = 23 |
| | Pink |
| 1. | $Ro = 0.60$; N = 2 |
| 2. | $\ln(Ro) = -14.603 + 2.668\ln(ATU/T) + 2.749\ln(T)$; $R^2 = 99.0$, N = 28 |
| | Sockeye |
| 1. | $Ro = 0.30$; N = 2 |
| 2. | $\ln(Ro) = -14.032 + 2.383\ln(ATU/T) + 2.866\ln(T)$; $R^2 = 98.5$, N = 30 |
| | Steelhead |
| 1. | $\ln(Ro) = -2.977 + 0.1969T$; $R^2 = 98.3$, N = 4 |
| 2. | $\ln(Ro) = -18.139 + 2.950\ln(ATU/T) + 4.549\ln(T)$; $R^2 = 97.9$, N = 28 |

totalling less than 1%), respectively. When at equilibrium in fresh water, at sea level, and at temperatures ranging from 0 to 24°C, $O_2$ and $N_2$ range in concentration from about 14.8-8.6 mg/l and 23.8-14.3 mg/l, respectively (Colt 1980). Excess total gas pressure or gas supersaturation can occur when air is entrained in water under pressure (i.e., air forced into solution in a plunge pool or injected via a Venturi-effect), when water is heated (since gas solubility decreases with an increase in temperature), or when biological or biochemical reactions occur such as photosynthesis (producing $O_2$) or denitrification of nitrate (producing $N_2$). During this state of supersaturation, gases will come out of

## TABLE 9

Critical oxygen concentration Pc (mg/l) as a function of accumulated temperature units (ATU), the accumulated temperature units from hatch (ATUa) and the ambient temperature (T) for three phases of incubation: (1) fertilization to completion of epiboly, (2) completion of epiboly to hatch, and (3) hatch to MAWW. $R^2$% and N (where sufficient data exists) have also been shown (from McLean et al. 1991).

| Phase | Equation |
|---|---|
| | Chinook |
| 1. | $Pc = 2.0$[1] |
| 2. | $\ln(Pc) = -3.302 + 0.976\ln(ATU/T) + 0.161T$; $R^2 = 91.5$, $N = 40$ |
| 3. | $Pc = 4.4$[2]; mean = 3.98, SD = 0.41, N = 46 |
| | Coho |
| 1. | $Pc = 2.0$[1] |
| 2. | $\ln(Pc) = -2.141 + 0.799\ln(ATU/T) + 0.128T$; $R^2 = 88.7$, $N = 41$ |
| 3. | $Pc = 5.586 - 0.664\ln(ATUa/T)$; $R^2 = 62.8$, $N = 38$ |
| | Chum |
| 1. | $Pc = 2.0$[1] |
| 2. | $\ln(Pc) = -3.634 + 0.976\ln(ATU/T) + 0.193T$; $R^2 = 88.9$, $N = 12$ |
| 3. | $Pc = 4.942 - 0.021\ln(ATUa/T)$; $R^2 = 49.9$, $N = 13$ |
| | Pink |
| 1. | $Pc = 2.0$[1] |
| 2. | $\ln(Pc) = -3.261 + 1.017\ln(ATU/T) + 0.131T$; $R^2 = 93.2$, $N = 14$ |
| 3. | $Pc = 4.2$[2]; mean = 3.65, SD = 0.55, N = 15 |
| | Sockeye |
| 1. | $Pc = 2.0$[1] |
| 2. | $\ln(Pc) = -2.214 + 0.693\ln(ATU/T) + 0.141T$; $R^2 = 90.1$, $N = 16$ |
| 3. | $Pc = 3.575 - 0.013(ATUa/T)$; $R^2 = 42.1$, $N = 12$ |
| | Steelhead |
| 1 & 2. | $\ln(Pc) = -4.50 + 0.949\ln(ATU/T) + 1.45\ln(T)$; $R^2 = 97.1$, $N = 20$ |
| 3. | $Pc = 5.56 - 1.04\ln(ATUa/T) + 0.131T$; $R^2 = 79.5$, $N = 20$ |

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[1] Data suggest that Pc is 2.0 mg from fertilization to completion of epiboly.

[2] No significant relationship exists with temperature, therefore the SD was added to the mean to give a conservative estimate of Pc.

**TABLE 10**

Mean alevin mortality, time to 50% hatch, and embryo to yolk dry weight ratios (E:Y) when sockeye salmon eggs were exposed to combinations of un-ionized ammonia ($NH_3$) and dissolved oxygen (DO) levels at 10°C and pH = 7.5 (from Jensen 1981).

| $NH_3$ ($\mu$g/l) | Alevin DO (mg/l) | Time to mortality (%) | 50% Hatch (°C-days) | E : Y[1] (mg:mg) |
|---|---|---|---|---|
| 10 | 10.9 | 0.6 | 649 | 0.294 |
| 10 | 3.4 | 1.9 | 660 | 0.163 |
| 191 | 10.9 | 1.4 | 649 | 0.264 |
| 191 | 3.4 | 38.1 | 713 | 0.117 |
| 100 | 3.0 | 54.7 | 714 | 0.114 |

[1] At 675 °C-days.

solution in the form of bubbles, both in water and in the organic materials in eggs and larvae causing gas bubble trauma. Jensen et al. (1986) assessed the response of juvenile salmonids to gas supersaturation and recommended "safe" levels ranging from 103.8-114.8% TGP, depending on associated factors of water depth, size of fish, and $O_2$:$N_2$ ratios. Alderdice and Jensen (1985) indicated that salmonid eggs have a relatively high tolerance to gas supersaturation ("safe" criterion of about 108-110% TGP) due to the compensating internal egg pressure.

*Ammonia.* Ammonia ($NH_3$) is the primary excretion product of teleost fish and is toxic at very low concentrations. Excess concentrations can cause mortality while sublethal levels can reduce growth and development rates (Jensen 1981). In combination, low DO and high $NH_3$ were shown to increase alevin mortality, delay hatching, and reduce yolk conversion (i.e., reduced embryo/yolk ratio) in sockeye salmon eggs (Table 10) exposed from fertilization to yolk absorption (Jensen 1981). Also, Jensen (unpubl. data) found coagulated yolk (white spot disease) in sockeye eggs exposed to a combination of low DO (3 mg/l) and high ammonia (100 mg/l $NH_3$); this response was absent when eggs were exposed separately to either low DO or high $NH_3$. Calamari et al. (1981) documented the changes in decreased tolerance of rainbow trout eggs from fertilization to the early fry stage as follows: 96-h $LC_{50}$ > 0.5 mg/l un-ionized $NH_3$ just after fertilization, about 0.15 mg/l at hatch, and about 0.1 mg/l at yolk absorption. Water chemistry strongly influences the toxicity of ammonia, since it is the un-ionized form

($NH_3$) that is toxic. The equilibrium expression is influenced most by pH and temperature. For example, an increase in pH from 7 to 8 increases the percent un-ionized ammonia by a factor of 10 (i.e., 0.19 to 1.8% $NH_3$ at 10°C), while an increase in temperature from 10 to 20°C increases the percent un-ionized ammonia by a factor of 2 (i.e., 0.19 to 0.40 at pH 7.0) (Emerson et al. 1975). In general, a high pH level will increase the toxicity of total ammonia by increasing the level of un-ionized ammonia.

*pH.* The adverse effects of acid rain are well documented in Europe and eastern North America, where severe reductions and elimination of salmonid populations have occurred because of extremely low pH levels in lakes and streams (Beamish et al. 1975; Farmer et al. 1980; Jensen and Snekvik 1972), usually because of recruitment failure. Laboratory studies (Menendez 1976; Daye and Garside 1977, 1979; Johansson et al. 1977; Peterson and Marin-Robichaud 1982) and field studies (Jensen and Snekvik 1972; Rosseland et al. 1980) indicate that sensitivity of eggs to low pH at fertilization and during embryonic and larval development is the main reason for the decrease in the anadromous salmonid populations in acid conditions. Low pH has also been shown to adversely influence oogenesis (Mount 1973; Craig and Baksi 1977; Ruby et al. 1977). Rombough (1983) exposed eggs and alevins of five Pacific salmon species and determined 10-d $LC_{50}$ and $LC_{10}$ values. The tests were conducted at 10°C and in very soft water (total hardness 11.4 mg/l as $CaCO_3$). He found eyed eggs to be more resistant (10-d $LC_{50}$; 3.6-4.0) than newly hatched (10-d $LC_{50}$; 4.4-4.9) and buttoned-up alevins (10-d $LC_{50}$; 4.4-5.2). The greater resistance of eggs to low pH is attributed to the egg capsule and the buffering capacity of the perivitelline fluid (Daye and Garside 1977, 1980; Johansson et al. 1977). Rombough (1983) cautioned that the tolerance of eyed Pacific salmon eggs to low pH may be an over-estimation, since other studies indicate that earlier stages of embryonic development (such as cleavage) are more sensitive to low pH (Daye and Garside 1977; Brown and Lynam 1981; Daye 1980). Kwain (1975) noted that rainbow trout embryos were more sensitive to low pH at lower temperatures, but attributed this to slower development and hence longer exposure time to low pH.

Low pH has also been shown to inhibit water uptake or perivitelline fluid formation in Atlantic salmon (Peterson and Martin-Robichaud 1982; Eddy and Talbot 1983) at pH 3.5 to 4.5. Peterson and Martin-Robichaud (1982) suggested that such impaired water uptake would make eggs more susceptible to mechanical shock. Rombough and Jensen (1985) investigated reduced water uptake and internal egg pressure in response to low pH on steelhead trout eggs. Their findings indicated a significant effect on egg pressure at pH 6.0 and lower. Their analysis also suggested that low pH interferes with cortical vesicle exocytosis, affecting osmotic activity of perivitelline colloids, and reduces capsule strength.

*Carbon Dioxide.* Carbon dioxide primarily is of concern to fish culturists because of its influence on the pH of the water. The concentration of $CO_2$ can be quite high in some groundwaters (>100 mg/l). In addition, $CO_2$ is the respiratory byproduct of animal metabolism, and hence concentration increases as a function of egg density in incubators.

Adverse effects of $CO_2$ are related to pH. Therefore, maximum acceptable $CO_2$ concentrations are generally based on minimum acceptable pH levels. To maintain pH near 7.0, the dissolved $CO_2$ should be about 20 mg/l or less (Sigma Resources 1983).

*Suspended Solids.* Suspended solids primarily occur in surface waters, while groundwaters generally, filtered by soil or rocks, are free of particulate matter. One exception is groundwater containing iron (ferrous ion), which precipitates (as ferric hydroxide) when aerated (ferrous ion oxidized to ferric ion). Such a precipitate could coat and smother developing salmon eggs. Maximum suggested concentrations of suspended solids for egg incubation is 3 mg/l (Sigma Resources 1983).

*Minerals.* Minimum hydromineral requirements during incubation of salmonid eggs presently are not well understood. Alderdice and Rombough (1987) suggested the following conservative estimates:

$Na^+$ ≥ 1 mg/l
$K^+$ ≈ 0.1 mg/l
$Ca^{+2}$ ≥ 10-11 mg/l
$Mg^{+2}$ no minimum requirement
Total water hardness (as $CaCO_3$) = 25 mg/l

Although, not directly lethal, exposure to suboptimal mineral levels can make eggs or alevins susceptible to pathogens such as documented by Alderdice and Harding (1987). The role of minerals during final maturation processes has also been shown (Lam et al. 1982) for chum salmon (that have lost their ability to osmoregulate), with better fertilization occurring when these fish were exposed to fresh water instead of salt water.

*Water Velocity.* The velocity of water flowing past developing eggs becomes important when many eggs are held in an incubation container at hatchery production loads, where oxygen can quickly become depleted, particulary as eggs near hatch. Hence, an adequate water velocity is essential for egg survival and embryonic growth. Silver et al. (1963) and Shumway et al. (1964) studied the combined effects of various oxygen levels (1.6 to 11.7 mg/l of dissolved oxygen) and water velocities (3 to 1,350 cm/h) on steelhead trout, chinook salmon, and coho salmon embryonic survival and growth. The lowest dissolved oxygen levels (1.6 mg/l) caused 100% egg mortality in two species tested, steelhead trout and chinook. In all species the size of hatching alevins was reduced and time to hatch increased at reduced velocities and dissolved oxygen levels. Brannon (1965) found that water velocities, ranging from 180 to 27,000 cm/h, had no effect on the growth of sockeye salmon embryos at hatch. A minimum apparent bulk velocity of 500 cm/h (equivalent to about 12 lpm flow in a vertical flow incubation tray) for incubation in salmon hatcheries is recommended.

### *Egg Handling Protocols*

Fish culturists have developed many innovative ways of handling eggs to enumerate and determine egg survival. Several descriptive books outline these procedures (Leitritz and Lewis 1976; Piper et al. 1982). Numbers of eggs at the time of fertilization are determined either by weight or volume, with small sub-samples counted to determine mean egg weight or egg volume. Eggs must be handled carefully, due to their sensitivity to mechanical shock (as mentioned above). The techniques developed for accurately counting egg sub-samples include egg-counting boards with small depressions and holes to allow liquid to drain through (Leitritz and Lewis 1976) or egg measuring troughs developed by von Bayer (1950). Measurement of total egg weight or volume must be carefully done, ensuring that excess water is minimized. Burrows (1951) developed a volumetric method of egg enumeration by measuring water displacement when eggs are added to a container.

At the eyed stage, when mechanical shock sensitivity is minimal, eggs are intentionally shocked by agitating the eggs enough to rupture the vitelline membrane around the yolk in dead eggs, but not so much as to damage normally developing eggs. This results in denaturation of the yolk protein and causes the dead eggs turn white. Hence, it is visibly easy to recognize dead eggs among the live eggs. Counting and removal of these dead eggs can be done manually with various types of modified forceps and siphon tubes, if the numbers of eggs is small. For large numbers of eggs, various types of automatic "egg pickers" (sorters) have been developed. There are automatic egg-pickers and counters that use photoelectric sensors to detect dead eggs due to the yolk denaturation, which affects light passage through an egg. Another method of removing dead eggs has utilized the different turgor pressures and elasticities of live and dead eggs. In this method eggs are directed off a ramp and fall freely onto an inclined platform, where the more turgid live eggs with elastic egg capsules bounce farther than do the dead eggs, which fall into a separate basket. The denatured protein in dead eggs also changes the density of eggs (live eggs have greater density than dead eggs). This principle also has been employed in separating live and dead eggs in various solutions of salt or sugar baths (Leitritz and Lewis 1976), where floating dead eggs can quickly be removed with a net or screen (this must be done quickly since the egg capsule allows passage of the salt or sugar solution into the dead egg, causing loss of buoyancy).

### *Disinfection of Eyed Eggs*

To prevent the transmission of diseases, salmonid eggs can be disinfected at the time of fertilization (either before or after water hardening with no appreciable difference in egg survival) or at the eyed stage. The chemical of choice is the iodophor Povidine, which is a complex of iodine that has a broad spectrum germicidal activity but is not as corrosive as elemental iodine. Some of the commercially available iodophors include Argentyne, Bridine, Ovadine, and Wescodyne; most contain 1-2% active iodine concentration. The recommended disinfection procedure (DFO 1984) is to prepare a 100 ppm (active iodine) in low organic content water (to minimize loss of free iodine) and adjust pH to 6.5-7.5 with 8% sodium bicarbonate, if necessary. Ensure that solution temperature is the same as the incubation temperature. Treat about 2,000 eggs per litre of disinfectant for a period of 10 min, followed by thorough rinsing. After disinfection ensure that eggs do not have contact with any contaminated or non-disinfected equipment or water. Iodophors are also routinely used to disinfect fish culture equipment, tanks, and fish culturist apparel such as boots and rubber gloves.

### *Fungicidal Treatments of Eggs*

In eggs, fungus infection (e.g., *Saprolegnia*) starts on dead eggs but can quickly spread to live eggs (Hoffman 1969; Amlacher 1970; Neish and Hughes 1980). The standard prophylactic or control fungicide used in hatcheries has been malachite green (Nelson 1974; Marking 1989). However, in both Canada and the U.S., malachite green is not authorized for use on food-fish (Nelson 1974; Dentler 1982; D. Kieser pers. comm.). Therefore, there is a great need to find a substitute for malachite green (Schnick 1988). The search for new fungicides has been extensive and well documented (Dentler 1982; Bailey 1983*a*, 1983*b*, 1984; Bailey and Jeffrey 1989; Schreck et al. 1991; Marking et al. 1994). These studies have found several alternative anti-fungal substances but most have been unsatisfactory; they are typically ineffective, expensive, labour intensive, unapproved or otherwise dangerous. Often these drugs were tested *in vitro* (Oláh and Farkas 1978; Bailey 1983*a*, 1983*b*), while other experiments show drugs to be effective *in vitro* but ineffective *in vivo* (Bootsma 1973; Schreck et al. 1991).

In British Columbia, alternative anti-fungal strategies have included salt baths (Edgell et al. 1993). Several treatment combinations of sea salt, NaCl, and $CaCl_2$ were tested on pre-eyed chinook salmon eggs. A solution of NaCl and $CaCl_2$ (20‰; at a ratio of 26:1 made with 0.7 g of anhydrous $CaCl_2$ and 19.3 g NaCl in 1 l water) applied for 1 h three times per week, was found to control fungus; 10‰ treatments were ineffective in fungus control and 25‰, although effective in fungus control, caused increased egg mortality. Taylor and Bailey (1979) controlled fungus on incubating pink salmon eggs by daily 2- to 3-h treatments with seawater.

Marking et al. (1994) evaluated the efficacy of 21 chemicals against pure fungal cultures of *Saprolegnia* and against *Saprolegnia*-infected rainbow trout eggs. Of the compounds tested, with a 60-min exposure every other day for about 2 weeks, formalin (at 500 and 1,000 ppm) actually decreased fungus infection, while hydrogen peroxide (at 1,000 ppm) and NaCl (at 30‰) controlled fungus. Some chemicals, although effective in controlling fungus growth, were rejected due to toxicity to eggs or to difficulty in obtaining regulatory approval. One chemical, iodine, rejected by Marking et al. (1994) due to toxicity to eggs, has been shown by Jensen et al. (in press) as elemental iodine (not as povidine) at concentrations of 0.2 to 0.5 mg/l to be effective in controlling fungus growth on developing salmon eggs.

### *Computer Programs for Fish Culturists*

In western Canada, microcomputers were originally installed in federal salmon hatcheries to improve financial and fish culture record keeping. Although data storage, organization and retrieval are still the main functions of microcomputers in these hatcheries, computers have also been used to assist hatchery workers with a wide range of fish culture problems. Programs have been developed that focus on incubation, freshwater rearing, water quality and fish health.

Models for predicting egg development rates, oxygen requirements and oxygen consumption rates for different species in production hatcheries were discussed by McLean and Lim (1985) and by Rombough (1986). Jensen (1988) first described an incubation program with predictive models for 25 embryonic stages (*see* Table 11) of development for six salmonid species (i.e., includes 150 different models). The models included in the program use Equation 7 (*see* page 323) to predict all stages except hatching, while Equation 8 was developed to predict time to 50% hatch. This program allows for quick calculation of development rates to various embryonic stages in response to constant (or mean) temperature.

New research information (i.e., the influence of egg size on fry emergence, Rombough 1985), development of Ro and Pc models for several embryonic stages (Rombough 1986; McLean et al. 1991), and the need to easily calculate development rates for fluctuating temperatures prompted the design of the mathematical models described above (McLean et al. 1991). Jensen et al. (1992) incorporated these models into a package of computer programs for Pacific salmon egg incubation and rearing called SIRP (Salmonid Incubation and Rearing Programs). The program consists of two main categories, namely (1) incubation and (2) rearing. The theory and model development for the incubation program modules are described in detail in McLean et al. (1991). Each module (for each of the six species) has similar mathematical relationships. Therefore, each module has the same required inputs [except for chinook slamon, which requires initial egg weight to determine emergence or maximum alevin wet weight (Rombough 1985)]. Hence, after choosing the species the user can simply input the spawning date, the number of eggs, initial mean egg weight (for chinook only), the number of Heath trays (or 1 if any other type of incubator), initial DO (in % saturation), BP (in mm Hg; this value is necessary to calculate mg/l DO), weekly mean incubation temperatures and water flow rates. The program then proceeds to calculate when egg and larval development stages will occur. In addition, oxygen consumption rate (Ro, mg of oxygen consumed by 1,000 eggs per h) and critical oxygen level (Pc, the point at which eggs, larvae or fry begin to exhibit dependent respiration) are also calculated for every weekly data set. Finally, the outflow DO values are calculated.

TABLE 11

Embryonic development in Pacific salmon and rainbow (steelhead) trout eggs from fertilization to fry emergence or maximum alevin wet weight (MAWW). The stages prior to hatch are subdivided into three phases: cleavage, epiboly and convergence, and organogenesis (from Velsen 1987).

| Stage | Description of stage |
|---|---|
| CLEAVAGE | |
| 1 | Bipolar differentiation: gathering of cytoplasm into a high mound at the animal pole; (bipolar differentiation also occurs in activated eggs that are not fertilized) |
| 2 | 2 cells (first cleavage); the first 5 divisions are in the horizontal plane only; shape and arrangement of individual cells (blastomeres) is regular |
| 3 | 4 cells |
| 4 | 8 cells |
| 5 | 16 cells |
| 6 | 32 cells; successive divisions occur in the horizontal as well as the vertical plane |
| 7 | Morula (mulberry blastodisc) with numerous small cells visible and the establishment of the periblast surrounding the morula; the morula changes into a high, nearly hemispherical mound with a cobbled surface, later to become a lower mound with more gradual slopes and a nearly smooth surface |
| 8 | Blastodisc flattening and starting to spread to nearly cover the periblast and the small oil droplets underneath it; posterior region of the blastodisc may start bulging |
| EPIBOLY AND CONVERGENCE | |
| 9 | Appearance of embryonic shield, germ ring and terminal node |
| 10 | ⅓ epiboly; germ ring ⅓ of the way toward total overgrowth of the yolk; neural groove on the embryonic shield |
| 11 | ½ epiboly; overgrowth of yolk half completed and germ ring at the equator; formation of axial strand and neural keel; first somites; Kupffer's vesicle |
| 12 | ¾ epiboly; germ ring ¾ overgrown; optic anlagen and 3 brain vesicles |
| 13 | Yolk plug less than head width; germ ring narrowing toward vegetal pole; blastoderm nearly covering entire yolk; otic and optic placodes |
| 14 | Yolk plug closed; yolk enclosed in cellular envelope (blastoderm) |

**TABLE 11 cont'd.**

| Stage | Description of Stage |
|---|---|
| ORGANOGENESIS | |
| 15 | Hindbrain (rhombencephalon) enlarging; trunk-tail mound raised but not undercut |
| 16 | Tail bud just free from yolk; one or two branchial pouches detectable; hindgut visible in side view |
| 17 | Heart beat; 1st branchial cleft formed; cloaca visible and free from yolk sac; metencephalon and myencephalon clearly distinct; head not undercut; spontaneous C-coil (movement) of trunk |
| 18 | ¼ of yolk surface vascularized; pectoral fin buds present; posterior half of body free from yolk sac; faint eye pigmentation |
| 19 | ⅔ yolk surface vascularized; pectoral fins disc-shaped; head undercut to jaw level; mouth not open |
| 20 | Eyes fully pigmented; yolk sac ¾ vascularized; head free and mouth open; cerebral hemispheres forming |
| 21 | Caudal flexing of the vertebral column; mesenchyme concentrations in caudal and anal fins; pectoral fins twitching |
| 22 | Operculum covers 1st branchial slit; dorsal mesenchyme present |
| 23 | Operculum covers all branchial arches; beginning of hatching; rhythmic breathing movements and frequent body wriggling and twitching; fin rays developing in anal, caudal, and dorsal fins |
| 24 | 50% hatch |
| 25 | Fry emergence; MAWW; or time for first feeding (ponding time in hatcheries) |

To illustrate the usefulness of these models Figures 17 and 18 were generated using SIRP at 8°C until hatching occurred. Note in Figure 17 the Ro values differ markedly for the six species, indicative of their different development rates. In Figure 18 the Pc levels also differ greatly depending on species. In addition, as the eggs near hatch, the outer egg membrane limits oxygen transfer to the developing embryo resulting in the highest Pc levels, followed by a rapid reduction in Pc values after hatch. Hence, fish culturists must ensure adequate water flow and DO levels as embryos near hatching. To summarize the typical range of Ro and Pc levels for the six salmonid species, SIRP was used to generate mean, minima, and maxima Ro and Pc levels at 4, 8, and 12°C (Table 12). Computer programs such as SIRP give fish culturists an extremely useful management tool, enabling one to quickly and accurately determine how long it will take for a given species of salmonid eggs to develop to a key developmental stage for any temperature regime. In addition, fish culturists can maintain "safe" DO levels while optimizing water usage by the careful control of water flows, temperatures, and egg loadings of the predicted weekly oxygen consumption and critical oxygen levels. "Safe" refers to DO levels with no negative effects on embryos. Finally, with the continuing

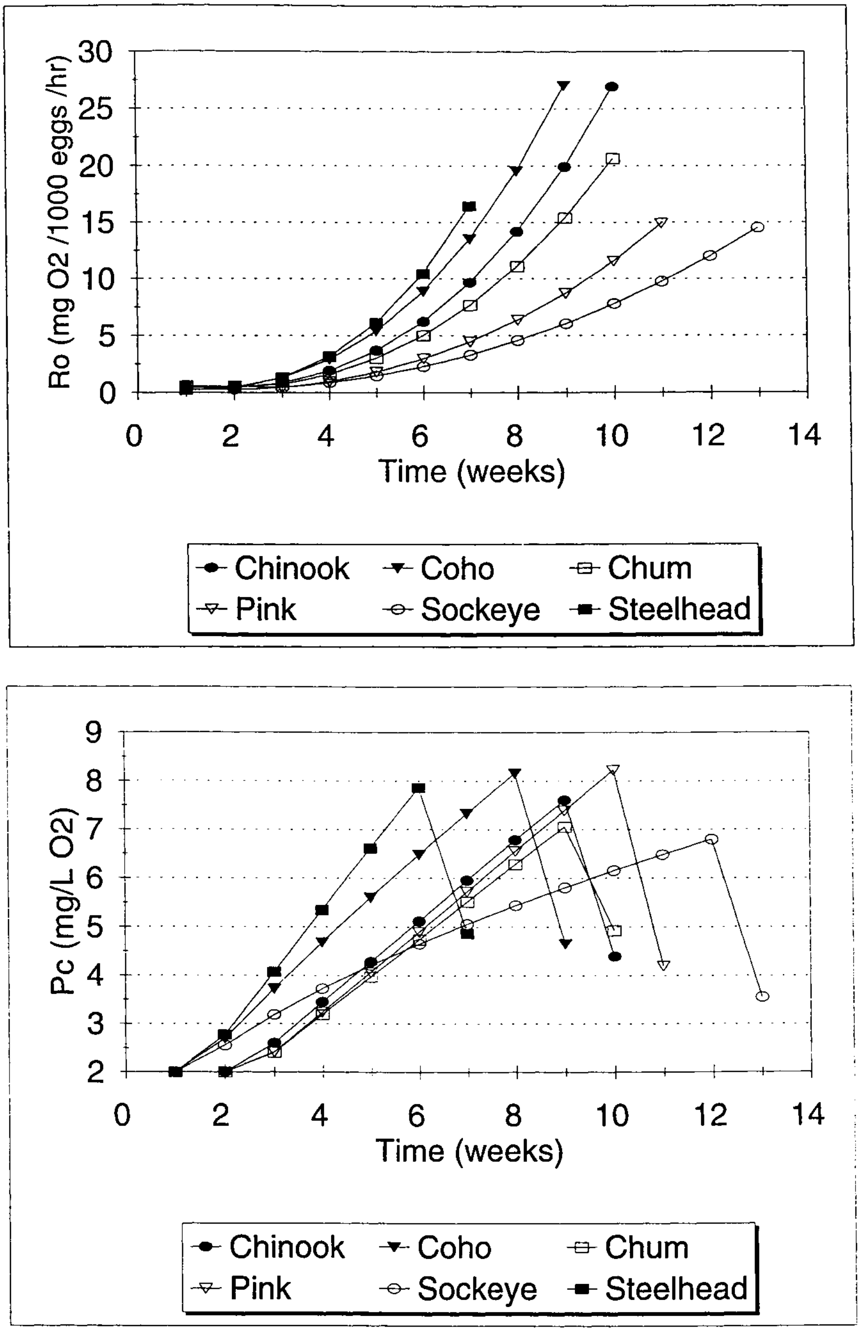

FIGURE 17. A. Oxygen consumption rate (Ro; mg $O_2$/1000 eggs/h) for five species of Pacific salmon and steelhead trout at 8°C. B. Critical oxygen points (Pc; mg/l $O_2$) for five species of Pacific salmon and steelhead trout at 8°C.

TABLE 12

Mean (minimum - maximum) oxygen consumption rates (Ro; mg $O_2$ /1000 eggs · $h^{-1}$) and critical oxygen concentration Pc (mg/l) for five Pacific salmon species and steelhead trout calculated from fertilization to hatch at 4, 8, and 12°C (calculations based on weekly predicted Ro and Pc values using the equations listed in Tables 8 and 9, respectively [N = number of weeks]).

| Species | Temperature | Ro (minimum - maximum) | Pc (minimum-maximum) | N |
|---|---|---|---|---|
| Chinook | 4°C | 3.0 (0.2 - 10.1) | 4.2 (2.0 - 7.4) | 18 |
| | 8°C | 8.4 (0.4 - 26.9) | 4.4 (2.0 - 7.6) | 10 |
| | 12°C | 15.4 (0.6 - 45.9) | 5.6 (2.0 - 9.8) | 7 |
| Coho | 4°C | 4.0 (0.3 - 13.4) | 5.0 (2.0 - 8.5) | 17 |
| | 8°C | 8.8 (0.5 - 27.0) | 5.0 (2.0 - 8.2) | 9 |
| | 12°C | 13.3 (0.6 - 37.1) | 5.8 (2.6 - 9.4) | 6 |
| Chum | 4°C | 3.0 (0.5 - 9.7) | 3.4 (2.0 - 5.7) | 17 |
| | 8°C | 6.6 (0.6 - 20.6) | 4.2 (2.0 - 7.0) | 10 |
| | 12°C | 9.7 (0.6 - 28.3) | 5.9 (2.0 - 10.3) | 7 |
| Pink | 4°C | 2.6 (0.6 - 8.3) | 4.6 (2.0 - 8.4) | 18 |
| | 8°C | 4.9 (0.5 - 14.9) | 4.6 (2.0 - 8.2) | 11 |
| | 12°C | 6.7 (0.5 - 19.5) | 5.4 (2.0 - 9.7) | 8 |
| Sockeye | 4°C | 1.8 (0.2 - 5.6) | 3.8 (2.0 - 5.6) | 20 |
| | 8°C | 4.9 (0.3 - 14.5) | 4.6 (2.0 - 6.7) | 13 |
| | 12°C | 6.8 (0.3 - 19.4) | 5.7 (2.0 - 8.5) | 9 |
| Steelhead | 4°C | 1.0 (0.1 - 3.4) | 3.4 (2.0 - 6.9) | 12 |
| | 8°C | 5.5 (0.2 - 16.5) | 4.8 (2.0 - 7.9) | 7 |
| | 12°C | 7.9 (0.3 - 20.0) | 5.3 (2.6 - 7.3) | 4 |

improvements in computer-based water quality monitoring equipment, the next step will be to use such models as described in SIRP to automatically control (via computer-control) incubation and rearing environmental conditions and hence improve embryo development, growth, and survival.

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# Chapter 6

# EARLY REARING

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## INTRODUCTION

### *Review of Problems Encountered in Fry Rearing*

Despite the long history of salmonid culture in fresh water and progress in technology and scientific research, there are elements of risk to which populations of cultured fish regularly fall prey. For example, a substantial number of fish fail to move onto a high plane of nutrition (and growth) after initiation of feeding; bacterial infection of gills, usually the result of sestonosis, the inflammation of gill tissue by exposure to suspended particulate (seston), is very common; fungal and bacterial attack of fins requiring frequent chemical treatment is also common; and some systemic diseases still cause high mortality under certain conditions. The failure of mechanical systems is another major cause of loss, although there are adequate remedies for this today. While fish density can be increased by addition of supplemental oxygen to water supplies or tanks, this can lead to a complex degradation of water quality, placing the entire production at risk.

Important new technologies have been introduced in recent years that have been of great value to freshwater rearing. There are many effective systems for managing dissolved gas levels, water temperature and disinfection, and to allow water re-use and operate alarm systems (*see* Senn et al. 1984; Colt et al. 1988). Computer technology now allows the collection and management of all hatchery data, and computers may be used to manage most of the hatchery's operations, from alarms to automatic feeders. Hatcheries run the gamut from small manual operations to large and automated facilities.

Overall, disease has always been and still is the largest proximate cause of production loss in freshwater hatcheries. Approaches to disease management vary, but they usually consist of two elements: the reduction or elimination of the pathogen, and the enhancement of the host's resistance to the pathogen, chiefly through controlling stress. Pathogen reduction is accomplished through choice of water source, water treatment, and general measures of hygiene. It is not always possible to distinguish between these two approaches; for example, most hygiene measures also have a positive effect on water quality, which reduces fish stress levels (*see* Chapter 13).

It is rare to completely eliminate pathogens from hatchery fish populations. Attempts to eliminate pathogens are usually made through legal restrictions to movement of fish designed to exclude non-native pathogens (e.g., DFO 1984; Carey and Pritchard 1991) or through very elaborate quarantine measures designed to certify introduced fish strains or species (Sippel 1983). However, the majority of pathogens frequently encountered in salmonid hatcheries are now enzootic and cosmopolitan. Some are transmitted vertically and these are very difficult to eliminate from an infected population. Others are so prevalent, for example *Saprolegnia* spp. fungi, that they are nearly impossible to eliminate from any facility.

Despite these difficulties, many waterborne and horizontally transmitted diseases can be virtually eliminated from culture facilities by using pathogen-free ground water and by using only disease free broodstock. The cost and availability of effective vaccines and other control measures will determine whether efforts to eliminate pathogens are cost effective.

Resistance to disease can be increased through stress reduction, which focuses on water quality, hatchery operations, and aspects of fish behaviour. A major stressor to fish is poor water quality which is usually a result of high suspended solid levels, high concentrations of ammonia, or low concentrations of dissolved oxygen. These factors can be controlled, but at a cost which may or may not be justified in terms of resulting productivity. Husbandry methods such as handling may also cause stress and this may be minimized, but never eliminated (Wedemeyer et al. 1990; Barton and Iwama 1991). Recent evidence shows that salmonids respond to stress within a few weeks after hatching (Barry et al. 1995).

Stress caused by behaviourial interactions between fish has long been recognized as a problem (Piper et al. 1982), but only recently has research and practice begun to look at both the range of behaviour and the effects of behaviour on stress levels; recent studies indicate that there is potential for reducing stress levels and increasing productivity through an understanding of fish behaviour (*see* Thorpe and Huntingford

1992). Environmental factors such as light intensity, water velocity and feeding method are known to interact with fish density to increase or reduce stress levels.

In other areas, research has reduced risks in fish production. Knowledge of salmonid nutrition has steadily increased, resulting in better fish health and growth under conditions of mass production. This is a significant contrast to days when liver bars, which are frozen preparations of beef liver, were used as a primary means to combat disease. The development of vaccines and therapeutic agents has increased resistance to disease, and broodstock programs have resulted in genetic improvements that improve fish growth and also reduce fish stress response to hatchery conditions (Pickering 1987).

Despite scientific and technological advances, there are many areas where fish culture practice goes on much as it did a century ago. Tank design has not been fully addressed by research, suspended particlulates pose serious problems in most rearing facilities, and the question of fitness of fish to be released into natural environments, while much discussed, remains largely unanswered. Although a knowledge of fish behaviour has important implications for fish rearing, comparatively few detailed studies of fish behaviour have been done under the crowded conditions of production rearing. In this chapter we attempt to summarize basic fish rearing practices, the scientific theory underlying these practices, and to highlight areas where there is a lack of understanding - the "loose ends" of fish culture.

## PONDING: THE INITIATION OF FREE-SWIMMING LIFE

### *Introduction*

The initial presentation of food marks the transition between incubation and rearing and is a critical stage in salmon culture. Fish culturists must not only decide when and how to present food but must also contend with the dramatic developmental and behavioural changes taking place at this time. Over a relatively short period, substrate-oriented, yolk-dependent larva (alevins) become free-swimming, externally feeding fry. The success of the subsequent rearing phase is dependent on satisfying these new requirements.

### *Transition from Alevin to Fry*

At hatch, salmonid alevins have a large yolk reserve. For example, chinook salmon (*Oncorhynchus tshawytscha*) alevins (wet weight basis) are approximately 70% yolk and 30% embryo (Heming 1979). The yolk-sac is delicate and sensitive to abrasion. Alevins are denser than water and in nature incubate deep in the gravel substrate of the streambed for protection and support. In a hatchery, alevins are seldom handled and are normally incubated in darkened containers. Often incubators are supplied with artificial substrate to simulate the gravel streambed.

As the embryo develops the yolk-sac is gradually absorbed. The disappearance of the visible yolk signals the "buttoning up" stage where the ventral slit closes. Even at this stage a significant quantity of yolk is hidden in the body cavity and continues to play an important role in alevin nutrition.

A high yolk conversion efficiency results in a large healthy fry at ponding. Yolk conversion efficiency depends on many factors. High levels of alevin activity induced by physical disturbance of incubators or high water velocity results in low yolk conversion efficiencies. These factors do not affect the rate of yolk utilization but reduce conversion efficiency and therefore lead to smaller fry at ponding (Brannon 1965; Murray and Beacham 1986).

Alevin activity can also cause yolk-sac malformation in Heath tray-type incubators (Emadi 1973). Swimming induced by high water velocity and lack of substrate results in abrasion of the yolk-sac on the incubator screen. Chum (*Oncorhynchus keta*), pink (*O. gorbuscha*) and sockeye (*O. nerka*) are more active than coho (*O. kisutch*) or chinook salmon. Yolk-sacs of affected alevins are elongated and often exhibit white spots of coagulated yolk. This condition results in high mortality later in development.

Exposure of eggs and alevins to diffuse light also has negative effects (Brannon 1965). Embryos are smaller at hatch and alevins attain swim-up sooner than those incubated in darkness. After emergence, alevins incubated in light are not frightened by overhead movement and probably are more vulnerable to predation. Yolk conversion is also reduced if oxygen concentrations are too low or alevins are exposed to poor water quality, e.g., high ammonia or gas supersaturation (Mason 1969; Rankin 1979; Rice and Bailey 1980; Jensen 1988).

Temperature and oxygen concentration not only affect conversion efficiency but also affect the rate at which yolk is absorbed. Higher water temperatures result in smaller fry and also more rapid yolk absorption (Alderdice and Velsen 1978; Heming 1982; Murray and McPhail 1988; Beacham and Murray 1990) whereas reduced oxygen levels lengthen the incubation period and result in smaller fry (Shumway et al. 1964; Mason 1969; Davis 1975).

As alevins metabolize yolk to meet energy requirements, their dry weight decreases over the incubation period. However their wet weight actually increases as yolk is converted into tissue. This occurs because tissue has a higher water content than yolk. The moisture content of tissue and yolk are 85% and 51% respectively so 1 g of yolk is converted into 3 g of tissue (Heming 1979). Alevin wet weight increases to a maximum value near the end of yolk absorption. Since maximum alevin wet weight (MAWW) occurs near the optimal ponding time, MAWW is an important concept in hatchery operations. The proportion of yolk at this stage is highly dependent on the incubation temperature. For example, chinook alevins increase from 4% yolk (96% tissue) at 5°C to 14% yolk (86% tissue) at 10°C (Rombough 1985).

Sometime near the attainment of MAWW, alevins abandon the bottom substrate and become free swimming (Heming 1982). To attain this neutrally buoyant or swim-up stage alevins swim to the water surface and fill their air bladder by swallowing an air bubble (Bams 1969). The timing of emergence is influenced by many environmental factors such as light, substrate type, temperature change and oxygen concentration and cannot be predicted solely from the age of the alevin. Precise timing of this stage is further complicated by the fact that newly emerged fry often re-enter the gravel a number of times before fully committing themselves to stream life. This tendency, although more pronounced when fry are frightened, is part of the general adaptation to free-swimming life (Bams 1979).

Exogenous feeding is also a poorly defined stage with respect to alevin development. Immediately after hatch the alimentary tract is blocked by a tissue plug and so exogenous feeding is impossible. When this clears early in the alevin stage external feeding can begin. Although some external feeding may occur at this stage, it does not play a significant role in the nutrition of the alevin. External feeding becomes critical later in the alevin stage when yolk reserves are exhausted (Heming et al. 1982).

### *Optimal Ponding Time*

Alevins ponded at the optimum time experience minimum mortality and maximum growth rates. If ponding is too early the alevin yolk-sac is exposed to abrasion and physical damage. If the damage is severe water penetrates the sac and causes yolk to precipitate. This results in the coagulated yolk syndrome and eventual death in early rearing. Also, early ponded alevins may not have attained neutral buoyancy and may still require rugose substrate. They often respond to their new rearing environment by crowding on the pond bottom and suffocating. If alevins at this stage are exposed to starter feed then the situation becomes even more critical. Starter food produces suspended solids in the water column which are very abrasive to newly developed gills (Banks et al. 1979). Early ponded fry are non-aggressive inefficient feeders so a high fraction of this food ends up fouling the environment. Ponding under these conditions usually results in heavy mortality.

If fry are ponded after their yolk reserves are completely exhausted the effects are just as detrimental. Such fry do not have sufficient energy reserves to learn to feed and starvation results. Fry trapped in incubation trays past emergence often suffer high mortality right in the trays (Heming 1979). Increased swimming activity results in physical damage and also increases the oxygen consumption rate. A combination of low oxygen stress and fungal invasion of damaged tissue usually results in heavy mortality in the trays.

Heming (1979) found that if chinook salmon were presented with food just prior to MAWW, fry weight continued to increase right through this difficult transition period. If feeding was started before or after this critical time fry weight either decreased or levelled off after ponding. Optimum ponding time resulted in heavier fry. Koss and Bromage (1990) found the same pattern with Atlantic salmon (*Salmo salar*). Alevins fed 65 ATUs (6.5 days at 10°C) prior to MAWW were larger than those fed after MAWW. There also was higher mortality when feed presentation was delayed more than 218 ATUs (3 weeks) after MAWW. However it was also noted that late initial feeding resulted in an immediate vigorous feeding response and those fry were more uniform during rearing.

Bams (1983) cautioned that optimum ponding time must be defined in relation to a specific rearing environment. Maximum growth potential may not be the most important factor for fry released into a natural setting or into a large rearing pond. In nature, immediate survival (predator avoidance, swimming ability) is more critical so fry emergence is usually later than the optimum time defined in terms of growth potential. In a large rearing pond, disease susceptibility and gill irritation from waste food have to be considered in defining optimum ponding time.

To pond fry successfully at production hatcheries, the temperature history of each batch of eggs is monitored and visual checks of the alevins are routinely made. These daily values are accumulated from the time of fertilization to give accumulated temperature units (ATU, °C-days) for a given egg take. ATUs required for optimum ponding are dependent on factors such as species, stock, temperature regime, egg weight and type of incubator and no single value can used for all hatcheries. Through experience, site specific values have been found that help the fish culturist decide when to pond. Along with the ATU record, visual checks are also made of the yolk-sac. Figure 1 shows a photograph of the ventral surface of a sample of large chinook alevins from the Quinsam Hatchery that were successfully ponded at 983 ATUs.

*Models to Predict Optimum Ponding Time*. A number of general models have been developed to predict optimum ponding time for salmonids. Jensen et al. (1992) assumed that ATUs to attain maximum alevin wet weight (MAWW) approximated the optimum ponding time. Data were obtained from various literature sources and time D (days) to MAWW was expressed as a function of average daily temperature T (°C) using the Bělehrádek model (Alderdice and Velsen 1978):

$$D = a / (T - c)^b \tag{1}$$

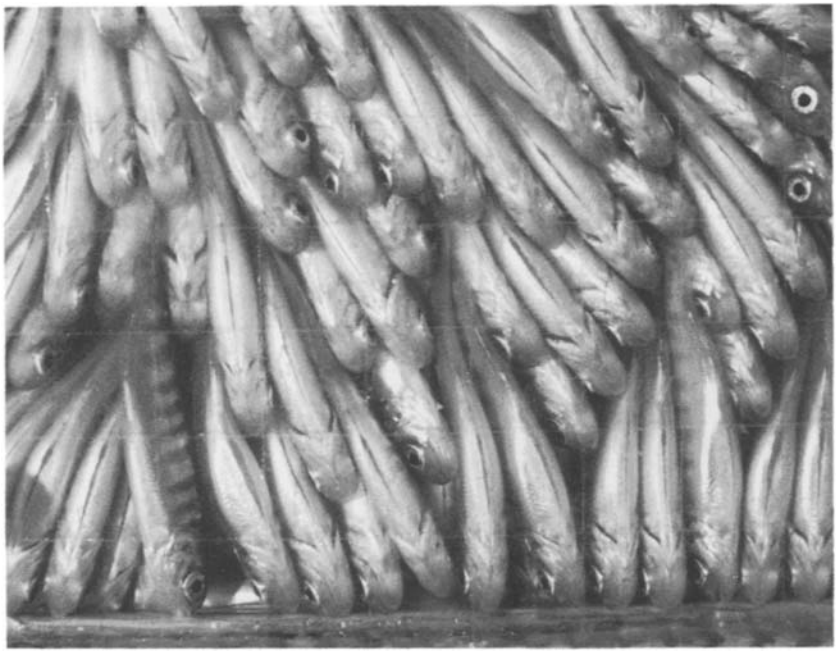

FIGURE 1. Ventral surface of chinook alevins that were successfully ponded at 983 ATUs.

Since ATUs are the product of average daily temperature and number of days, ATUs to ponding is given by:

$$ATU = T\ D = T\ a\ /\ (T - c)^b \qquad (2)$$

where the constants a, b, and c are characteristic of a species.

Constants were derived for six species of salmonids (McLean et al. 1991). The size N and temperature range of the data sets used to derive the constants were as follows: chum N=11, 3.9 to 13.9°C; sockeye N=31, 1.1 to 13.9°C; coho N=16, 2.3 to 13.9°C; pink N=7, 3.0 to 13.9°C; steelhead (*Oncorhynchus mykiss*) N=3, 6.0 to 15.1°C; chinook N=26, 2.3 to 13.9°C. Constants for various species are shown in Table 1. These models were used to predict ATUs to MAWW as a function of the average incubation temperature (Figure 2).

Beacham and Murray (1990) explored a number of predictive models and also found the Bělehrádek equation a suitable predictor of ATUs to emergence. These

**TABLE 1**

Constants for predicting ATUs to MAWW using Equation 2 (McLean et al. 1991).

| Species | a | b | c |
|---|---|---|---|
| Chinook | 52409077.4 | 3.92184502 | −19.07204758 |
| Chum | 314.41470 | 0.51820254 | 1.6820473 |
| Coho | 923367.454 | 2.8995311 | −15.02834151 |
| Pink | 977660.461 | 2.7231174 | −18.83916220 |
| Sockeye | 34186861.2 | 3.56109377 | −24.96801241 |
| Steelhead | 922049.74 | 3.00725581 | −14.19575994 |

models however were based on larger data sets: chum N=112; sockeye N=106; chinook N=53; coho N=92; pink (odd year class) N=58; pink (even year) N=54. Constants for various species are shown in Table 2. Predicted ATUs to emergence are plotted against the mean incubation temperature in Figure 2.

Rombough (1985) derived an optimum ponding time model for chinook salmon based on experimental work with eggs obtained from the Big Qualicum river on Vancouver Island. Eggs were sorted into different size classes [mean water hardened weight (mg) ± standard deviation SD; 163±7; 235±10; 281±9; 340±9; 341±36; 384±27; 425±11 and 437± 8] and incubated at constant temperatures of 5, 7.5, 10 and 12.5°C. Wet weight was closely monitored and times to MAWW were obtained. It was assumed that optimum ponding and MAWW coincided. Time to MAWW was affected by temperature in a predictable way but was also significantly affected by initial egg weight. Larger eggs produced larger alevins that took longer to attain MAWW. ATUs to MAWW were related to temperature T and initial egg weight W by:

$$\text{ATU} = T \exp(5.88 - 0.152\,T + 0.000513W) \tag{3}$$

Note: $\exp(x) = e^{x}$, $e = 2.718...$

Predicted ATUs to MAWW at egg weights of 280, 360 and 460 mg are shown as a function of temperature in Figure 2.

Heming (1979) obtained chinook salmon eggs from Quinsam Hatchery on Vancouver Island and incubated them at constant temperatures of 6, 8, 10 and 12°C. These eggs were very large with a mean water hardened weight of 490 mg. Food was presented to alevins at various stages of yolk absorption and mortality and growth rates in early rearing were monitored. Lowest rearing mortality and maximum biomass was

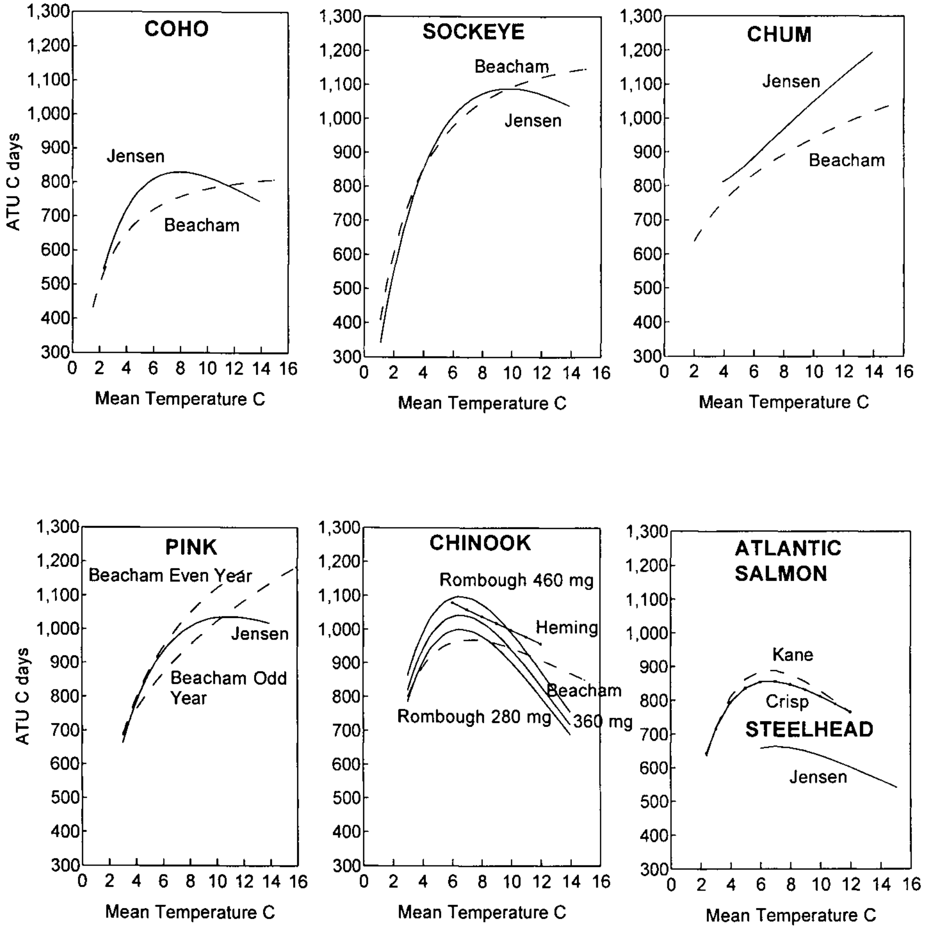

FIGURE 2. ATUs to ponding for various species as a function of the mean incubation temperature. Predictions are based on Heming 1979; Rombough 1985; Crisp 1988; Kane 1988; Beacham and Murray 1990; Jensen et al. 1992. Rombough's predictions for chinook are shown for eggs weighing 280, 360 and 420 mg.

achieved when food initiation occurred just before MAWW. Fry fed at the optimum time continued to increase in weight during the transition from incubation to rearing. When feed initiation occurred after the optimum time, fry weights showed a leveling off or even a decline during the transition to rearing. Mortality and final fry weights during early rearing (to 1758 ATUs) for the 10°C treatments are shown in Figure 3. From this work it was concluded that for chinook, the optimum ponding time occurred just prior to MAWW and that MAWW decreased from 1112 ATUs at 6°C to 950 ATUs at 12°C. At

TABLE 2

Constants for predicting ATUs to emergence using Equation 2 (Beacham and Murray 1990).

| Species | a | b | c |
|---|---|---|---|
| Odd year pink | 509.28 | −0.693 | 0.239 |
| Even year pink | 1381.60 | −0.967 | −3.446 |
| Chum | 548.40 | −0.764 | −0.039 |
| Chinook | 32991.33 | −2.043 | −7.575 |
| Coho | 1116.55 | −1.069 | −2.062 |
| Sockeye | 2094.35 | −1.134 | −3.514 |

10°C for example, MAWW occurred at 1045 ATU whereas the optimum time for feed initiation occurred 4 days earlier. The optimum number of ATUs was related to average temperature T by:

$$ATU = 1201.1 - 20.3\ T \tag{4}$$

These values are compared with other predictive models in Figure 2.

Kane (1988) related optimum ponding time to incubation temperature for Atlantic salmon. The effect of 13 different seasonally varying temperature regimes on development rate were observed. Ponding and exogenous feeding were assumed to occur when the yolk weight of formalin-preserved alevins was 5% of the total alevin wet weight. ATU to ponding was related to the average incubation temperature T by:

$$ATU = T \exp(6.003 \exp(-0.0307\ T)) \tag{5}$$

Crisp (1988) found that the time to swim-up for Atlantic salmon (D3) was related to hatch time (D2) by: $D3 = 1.7 * D2$. Time to hatch was related to the average incubation temperature by a power function: $D2 = 155167\ (T+11)^{-2.6562}$ (Crisp 1981). The relationship was derived for 10 temperature regimes whose means varied between 2.4 and 12°C. D2 was substituted into D3 and multiplied by T to predict the ATUs to swim-up:

$$ATU = 1.7\ T\ D2 = T\ 263784\ (T + 11)^{-2.6562} \tag{6}$$

Equations 5 and 6 have been plotted against temperature in Figure 2.

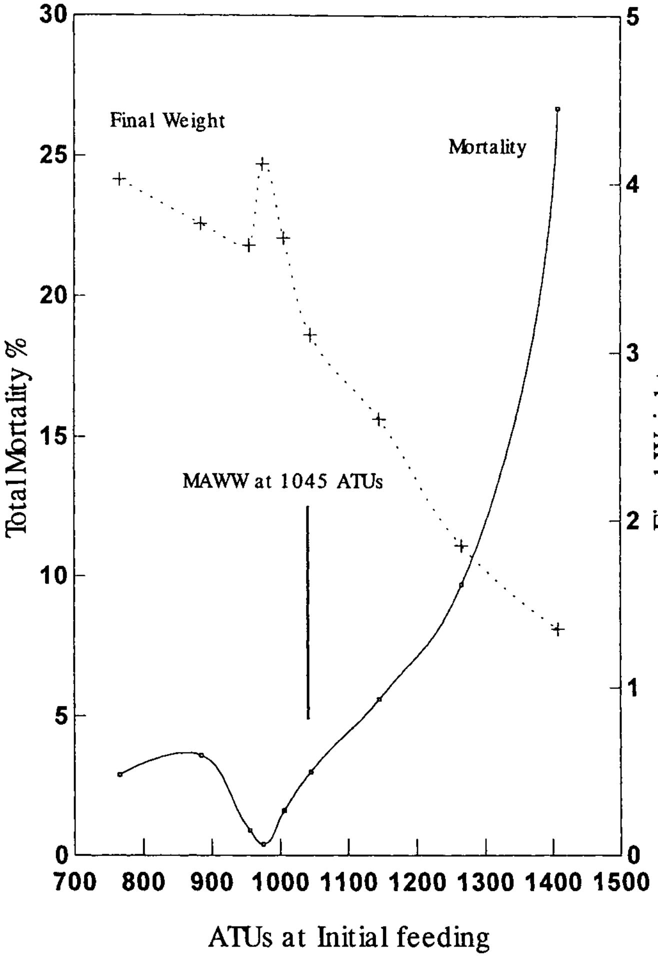

FIGURE 3. Performance of chinook fry over early rearing stage as a function of ponding time. Mortality and final weight of fry at the end of the study period are plotted against the ATUs at which fry were ponded and presented with food (Heming 1979). Lowest mortality and highest final weight occurred with fry ponded just prior to MAWW.

*Computation Methods.* If incubation temperature (T) is constant, the number of temperature units to ponding (ATU) is predicted by substituting T directly into the predictive equations. Time to ponding in days (D) is calculated from D = ATU/T. If temperature varies over the incubation period an estimate of the average incubation temperature can be substituted in the equations to give a first approximation of ponding time.

A more accurate method for variable temperature regimes involves calculating the daily rate of development (percent per day) from the average daily temperature. Ponding should occur when the sum of the daily development rates reaches 100% (Kane 1988). This method was also used by Alderdice and Velsen (1978) and Crisp (1988) to predict hatch times.

Daily development rates (R) for coho salmon are shown (Table 3) for average daily temperatures (T) between 1 and 14°C. This example is based on the time to emergence (D) model of Beacham and Murray (1990) (*see* Equation 1). In this case $D = 1116.55\ (T + 2.062)^{-1.069}$ and the daily percent development $R = 100/D$ (Table 3).

If the temperature regime consists of equal number of days at 4°C and 14°C (average incubation temperature = 9°C) the approximate number of days to emergence is found by substituting 9°C into the equation for D or by using Table 3, $D = 100/R = 100/1.169 = 85.5$ days. Summing development rates for equal numbers of days (N) at 4 and 14°C gives a more accurate predicted value of: $D = 2*N = 2*100/(0.615 + 1.742) = 84.9$ days. Although the difference is small in this example, summing development rates is recommended for surface waters where temperature is variable. In this case projecting the average daily temperature over the coming incubation period is difficult and daily summation of development rates is required.

*Model Limitations and the Importance of Site Specific Factors.* Predictive models based on laboratory tests or derived from large diverse data sets must be applied cautiously in a production setting. They provide a first approximation that must be tested by experience. Several studies are presented which show how site specific factors complicate the prediction of optimum ponding time.

The implication of egg size variation on the operation of production hatcheries has been investigated by Austin (1989). Rombough (1985) (*see* Equation 3) showed that initial egg weight had an important effect on ponding time for chinook salmon but did not explain how to deal with egg size variation in large scale fish culture. Austin (1989) measured egg size variation at Quinsam Hatchery (near Campbell River, BC). Figure 4 shows the distribution of mean eyed egg weights for 68 females spawned in the fall of 1989. Fish were 3 to 5 years old. Mean egg weights varied from 262 mg to 559 mg — individual egg size variation would be even greater. At an average incubation temperature of 9°C, Equation 3 predicts that alevins from small and large eggs would reach MAWW at 938 and 1092 ATUs respectively. This represents a range of 17 days so ponding according to the medium size would be 8.5 days too late for the small and 8.5 days too early for the large group. To reduce this discrepancy, a machine was developed to sort large numbers of eggs at the eyed stage into small, medium and large categories. Fry from these three size classes were ponded at their optimum times so that performance in early rearing was maximized. Competition between fry was reduced as fry learned to feed and mortality rates were reduced compared to unsorted groups.

TABLE 3

Daily percent development versus temperature for coho salmon.

| T °C | R %/day | T °C | R %/day |
|---|---|---|---|
| 1 | 0.296 | 8 | 1.057 |
| 2 | 0.401 | 9 | 1.169 |
| 3 | 0.507 | 10 | 1.283 |
| 4 | 0.615 | 11 | 1.397 |
| 5 | 0.724 | 12 | 1.511 |
| 6 | 0.834 | 13 | 1.627 |
| 7 | 0.945 | 14 | 1.742 |

Although Equation 3 showed the general trend between egg size and ponding time, it was not a useful predictor of ponding time at Quinsam Hatchery. Austin (1992) used size sorted chinook eggs incubated at nearly constant temperatures to test the accuracy of various ponding time models in the production setting. Large eggs were incubated at between 9 and 10°C in vertically stacked Heath trays (5500 eggs per tray). These incubators have smooth walls and lack substrate. Equation 3 was used to estimate optimum ponding time. Fry were ponded at the predicted optimum time and also one week before and one week later. Ponded fish were monitored for growth and mortality for the first 30 days of rearing. The test was carried out using 1990 brood chinook and was repeated again in 1991. These groups were incubated at mean temperatures of 9.5 and 9.8°C and had mean eyed egg weights of 474 mg and 513 mg respectively. The predicted times to MAWW (Equation 3) were 1025 and 1029 ATUs. In both years fry ponded one week early had significantly lower mortality than fry ponded at the predicted MAWW. Fry ponded one week after MAWW were in very poor condition and had very high mortality in early rearing.

These data show that optimum ponding time at this facility could be as much as 70°C ATUs before the MAWW predicted by Equation 3. These observations show that at these tray loads and water temperatures fry deteriorate very quickly if held too long in Heath trays. Fry held to MAWW showed clear signs of fin damage and physical abrasion. These fish were actively swimming and were probably injured as they attempted to escape the confines of the Heath basket. This example shows that site specific factors (type of incubator, loads) play an important role in determining optimum ponding time. At this facility the advantage of ponding at the predicted optimum time were overridden by adverse conditions that developed in the incubator.

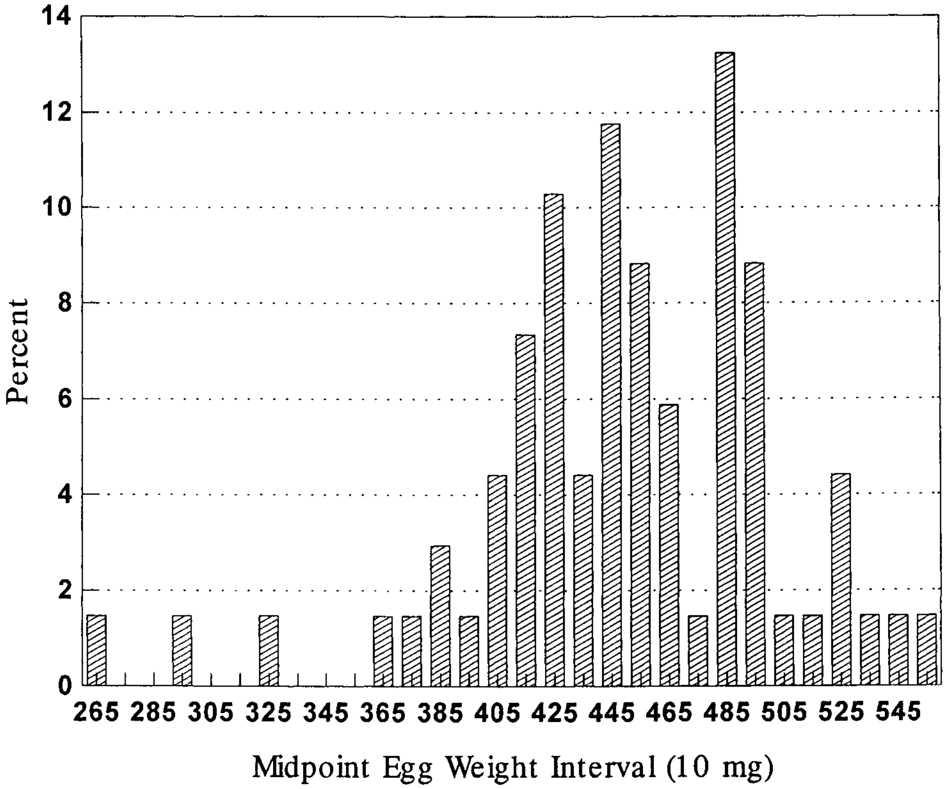

FIGURE 4. Frequency distribution of mean egg weights (eyed) of 68 chinook females (Austin 1989).

Bams (1970; 1979) studied up-welling incubators filled with gravel substrate where fry emerged and migrated on their own volition. Migration timing and fry quality from these incubators were compared to natural migrations. These studies were carried out on both pink and chum salmon. With identical temperature regimes, fry migrated from the gravel incubators 1 to 2 weeks prematurely. Incubator fish migrated at an earlier stage of development — indicated by having a shorter length and higher developmental index $K_D$ (note $K_D = 10*W^{1/3}/L$ where W = weight in mg and L = fork length in mm). Pink fry leaving gravel boxes on Hooknose Creek had a mean of 1.933 while fry migrating naturally had a $K_D$ of 1.8856. This 0.047 difference was statistically significant and consistent over the entire migration. The same pattern, although slightly less pronounced, was observed with chum. This phenomenon was considered to be an artifact of upwelling gravel incubators (Bams 1979). In nature, newly emerged fry often re-enter the gravel where they develop further before fully committing themselves to the stream. In gravel incubators, newly emerged fry are often swept to holding containers and so are forced to migrate.

In spawning channels, an optimum number of adults spawn naturally in clean graded gravel and are provided with optimum water flows during incubation. At emergence fry migrate from the channel volitionally. Lewis (1991) monitored the migration of chum fry from the Little Qualicum spawning channel in the spring of 1991. Fry migrating out of the channel between late March and early May were routinely sampled and measured for individual length and weight, yolk reserves and seawater tolerance. Early migrating fry had large yolk reserves (8%) and were unable to tolerate seawater (96-h $LC_{50}$ = 19‰) and were judged to be poor quality. This corresponded with the observations of fish culturists that the early fry seemed weak and that they could not be handled without high mortality. Fry migrating after April 27 had a lower yolk content (2%), were much more tolerant to seawater (96-h $LC_{50}$ = 35‰) and could tolerate being handled. These later fry were of much higher quality.

It is likely that poor environmental conditions forced a number of fry to migrate early. Areas of the channel had been smothered by silt resulting in reduction in intragravel water flow. This resulted in low oxygen and high ammonia concentrations which would have become critical as the metabolic rate of the alevins increased. This resulted in the early emergence of fry of very poor quality. In this example, a degraded incubation environment had an overriding effect on the timing of fry emergence.

### *Ponding Techniques*

*Rearing Pond Net Liners.* Even if alevins are transferred from incubators to large rearing units at the optimum time, difficulties can arise over the next few weeks. As discussed in the previous section, alevins do not develop at the same rate and so a particular ponding date is a compromise. More slowly developing alevins suffer the most drastic effects. They are not yet free swimming and crowd together on the bottom of the pond and suffocate. This occurs because concentrations of uneaten food and feces are high on the pond bottom and the horizontal water flow along the pond floor cannot supply the mass of alevins with oxygen. To reduce this problem alevins can be ponded into netliners that are floating in the rearing ponds. Fry can swim off of the liner into the rearing pond when they are ready. Alevins with slower development are kept off the pond bottom and held in an adequate flow so their oxygen demands can be met while they continue to develop. The netliner is also an effective way of removing dead or malformed alevins before they foul the rearing environment.

*Delay in First Feeding.* Forrest and Halley (1988) ponded chinook salmon fry from Heath trays prior to MAWW but delayed initial feeding for up to 14 days. It was hypothesized that a delay in initial feeding would be advantageous. This period would allow fry to adjust to their new environment while they lived off their internal yolk reserves. It was surmised that after this period of adjustment they would feed more aggressively and less feed would be wasted to foul the water and irritate gills. Chinook fry ponded at a water temperature of 7°C and then starved for up to 14 days (1154

ATUs) did not show increased mortality during rearing. This was not the case for fry held an additional 14 days in Heath trays — these fish suffered much higher mortality during rearing. Fish ponded at MAWW and starved for a period showed a more aggressive feeding response, lower mortality, slightly higher specific growth rates and a lower coefficient of variation. After 60 days of rearing the highest biomass was achieved by groups delayed between 4 and 7 days. A similar tendency was also noted with Atlantic salmon fry (Koss and Bromage 1990). In this study the greatest weight was attained by fish fed prior to MAWW; however, delayed feeding caused a vigorous feeding response which resulted in a lower coefficient of variation during rearing.

*Water Flow Required for Newly Ponded Fry.* Newly ponded fry are usually fed a special diet higher in fat and protein. Starter diets have a very small particle size and uneaten food either floats or stays in the water column for a period before sinking to the pond bottom. Fry are presented with high ration levels while they learn to feed and because they are inefficient feeders much of the food is wasted. This leads to high concentrations of suspended particulate material in the water column. This material has a high protein content and quickly begins to decompose and produce ammonia (Peel 1983). This is a critical environmental stressor for newly ponded fry and can cause gill mucous proliferation and bacterial gill disease (Banks et al. 1979).

Water flow requirements for rearing are discussed later (*see* page 403). Flows are set to maintain acceptable levels of dissolved oxygen and metabolites in the rearing pond. However to address the special concerns of newly ponded fry, Banks et al. 1979 have developed flow criteria based on suspended solids. To keep exposure to suspended solids at an acceptable level in a rearing pond, the ratio of daily food input (g) to water flow (l/min) must be less than 9 g food per l/min. If 1 kg of food is presented per day, the pond flow must be at least 1000/9 = 111 l/min. This is almost twice the flow required to meet the most stringent dissolved oxygen criterion (*see* Table 4).

## FEEDING AND REARING

There are both differences and similarities between the feeding strategies suited to wild and cultured fish. The wild fish forages in a variety of environments, usually in either the open pelagic zone or in fast moving water. In pelagic environments, food is encountered unpredictably, and fish schooling behaviour is common while territorial defense is generally not useful and not noted. In moving water such as streams and rivers, the holding of defended territory is the rule since food comes predictably from a source to potential fish positions (Wankowski and Thorpe 1979; Metcalf et al. 1992; Noakes and Grant 1992). Territoriality frequently results in dominance hierarchies expressed as size differences in such populations (Kalleberg 1958; Symons 1968). Many species shift habitats with changes in life stage (e.g., all smolting salmonids) or seasonally [e.g., coho salmon moving from streams to wintering ponds (Brown and Hartman 1988;

and *see* Chapter 2)]. Some of these fish behaviours may be innate and species specific while others may be modifiable by the environment; in either case they will be very important to fish culturists.

There is a considerable literature on the behaviour of salmonids in natural or simulated natural environments (e.g., Kalleberg 1958; Hartman 1965; Fenderson et al. 1968; Hartman and Gill 1968; Symons 1968; Dill et el. 1981; Abbott 1985; Abbott et al. 1985; Abbott and Dill 1989), and there is a body of general ecological theory on optimum foraging strategy that is applicable to fish (e.g., *see* Knights 1985; Townsend and Winfield 1985; Hart 1993). In essence, carnivorous fish require an optimum particle size and energy content to profit (to maximize energy) from hunting activity. For example an optimum prey weight of 0.01% - 0.1% of fish body weight has been suggested by several investigators (reviewed by Pandian and Vivekanandan 1985). Optimum prey size will also vary with distribution and abundance of prey and its energy content. The amount of prey consumed will be limited by gastric evacuation rate which mediates appetite (Brett 1971*a*; Ruggerone 1989), and which is largely a function of digestibility, fish size, and temperature, but is also affected by meal size and the nature of the feed intake (e.g., one large meal taken into an empty gut vs food taken into a partially filled gut (e.g., Talbot et al. 1984; *see* discussion below)).

Many wild salmonids also display both seasonal and diurnal feeding patterns often related to prey availability. Sockeye salmon for example, perform diel vertical migrations in lakes, which may be due to zooplankton migrations, predation avoidance, energetic considerations and vertical temperature distribution, or to some combination of these (Brett 1971*b*; Clark and Levy 1988; Levy 1990). Stream dwelling salmonids often show crepuscular feeding patterns related to the availability of drift insects in streams (Eriksson and Alanärä 1992; Mundie et al. 1990). Coho and Atlantic salmon show pronounced seasonal feeding and growth patterns related to both photoperiod and temperature. Coho salmon fry, for example, virtually "hibernate" (live on stored reserves and show very little activity) during winter months (Brown and McMahn 1987; McMahn and Hartman 1989) as do Atlantic salmon parr (Jansen and Johnsen 1986; Cunjak 1988; Metcalf et al. 1992). During these periods there is little attempt at feeding, and in the case of coho fry, shelter is sought presumably to avoid predators and wash-out by freshet.

In contrast to fish living in natural environments, hatchery fish must live in a very homogeneous surrounding and contend with densities orders of magnitude greater than those in nature. In contrast to diverse living food, hatchery salmonids are fed a homogeneous, non-living diet on a regular, but often arbitrary schedule, usually in far greater amounts than would be experienced in nature. Salmonids always prefer live foods to formulated feeds (pellets), and very quickly learn to feed effectively when released from hatcheries (e.g., Johnsen and Ugedal 1986), but they are easily trained to eat formulated feeds, at least if they have not experienced live feeds.

High feeding rates and crowding in the hatchery also alter water quality parameters from those usually expected in nature. Water quality is usually reduced in comparison with the natural environment, typically yielding lower concentrations of dissolved oxygen, and higher concentrations of ammonia and organic seston. Although stress levels may be high, there are few encounters with predators. There are several important issues which these distinctions bring to light: Can hatchery fish released into wild waters adapt to natural conditions and attain high performance in growth and survival? Do the rather different conditions in the hatchery have negative effects on fish? How much of the observed wild behaviour is innate and must be accommodated in the hatchery, and how much can be overridden by hatchery strategy?

Feed size, shape and energy content, and to some extent, palatability, can be controlled, as can daily ration (plane of nutrition), mode and time of presentation, and frequency of feeding, but are all of these significant factors in fish production? The usual hatchery goals are to produce healthy, rapidly growing fish of similar size (low coefficient of variation), with low stress levels, and with intact fins. This should be done at as low a cost as possible. There is also concern when fish are released, that they are able to adapt hatchery feeding behaviour to capturing diverse wild feeds, and to avoid predators. Although relatively little formal research has been done to determine the best hatchery protocols with respect to all of these issues, a growing body of literature is beginning to illuminate them. Hatchery traditions based on experience, common sense and informal trials have developed over many decades (e.g., Piper et al. 1982; Rutherford 1992). Many of these traditions appear to be effective.

### *Hatchery Protocols for Fry and Fingerling Feeding*

Obtaining high growth rates requires a high ration level (daily intake of food), and although feed constitutes a major cost in fish culture operations, small fish have high specific growth rates, high feed conversion rates, and, because of their small size, eat relatively little when compared to larger fish during on-growing (the rearing of fish to larger sizes suitable for harvest). It is in the early stages of rearing that one should feed at maximum ration levels to achieve the highest growth rates at least cost. Specific feed rate (as percent of the body weight fed per day) varies inversely with fish body weight, inversely with feed energy content, and directly with temperature (Brett and Groves 1979). The relationships between maximum ration and both fish size (weight) and temperature are log-linear; as fish become larger, they eat a lower proportion of their body weight (specific feed rate) but rate of decrease with weight declines with increasing fish weight to an asymptote. Smaller fish have a larger relative stomach capacity and a faster gut evacuation rate that is in accord with their higher metabolic rates and therefore energy needs (Brett 1979; Brett and Groves 1979). The relationship of feed rate with temperature is concave upward (*see* Stauffer 1973 for review). Hatchery feed charts have been developed from these relationships, many of them based on early work done by

Haskell (1959). Stauffer (1973) developed a theoretical feed rate model for maximum ration which is used today in many Pacific Northwest hatcheries.

Although such charts suggest an average maximum ration for fish of different sizes and for different water temperatures, they frequently suggest less than fish could eat. When different charts are compared they often vary widely. Since feed energy content has an inverse relation to maximum food intake, feeds of different energy content should require different feeding tables (e.g., Buterbaugh and Willoughby 1967; Hilton and Slinger 1981; Cho 1992). Feed tables are best seen as guides, and very significant departures from them can be observed. Other methods of estimating feeding rates are available based on hatchery constants and expected growth and conversion rates as seen in previous hatchery data (e.g., Piper et al. 1982; Westers 1987). Attaining a high plane of nutrition is related to appetite, a complex phenomenon involving endocrine responses, gut clearance rates, and to feed pellet acceptance. Pellet acceptance is related to organoleptic properties, size, shape and texture of food (e.g., Brett 1979; Millward 1989; Cho 1992; and discussion below).

Once a desired ration has been determined the number of feedings per day must be considered, and this itself affects plane of nutrition. Brett (1971*a*) and Shelbourn et al. (1973) investigated the effect of feeding strategy on total daily intake of sockeye salmon fingerlings. In both studies appetite (indicated by amount of food eaten) was shown to be related to emptiness of the stomach. The maximum ration set by a single satiation feeding (fish fed one meal slowly, over 40-50 minutes, until unable to accept additional food) was far less than a ration fed twice a day, with the first meal about two thirds the amount of a satiation feeding, and the second meal, at the end of the day, offered to satiation (Brett 1971*a*). This was explained by stomach clearance such that the smaller initial feeding allowed the stomach to clear to a greater extent by the time of the evening feeding, thereby increasing appetite maximally. Three meals per day resulted in still higher intakes (Brett 1971*a*). Shelbourn at al. (1973) also found that very small sockeye fry fed continuously attained significantly higher growth rates than fish fed three times per day to satiation. These studies were done at relatively high temperatures; gut clearance times are much longer at low temperatures. Although fish to some extent eat to attain an energy requirement (*see* Chapter 10, and Brett and Groves 1979; Jobling and Wandsvik 1983; Majid 1986; Smith 1988; Cho 1992), their intake can be modified considerably by feeding strategy.

Although a maximum ration may be desired at this stage of rearing, overfeeding reduces assimilation rate and may contribute to water quality problems including ammonia production and circulating fines (seston), which irritate gills. Overfeeding becomes a serious financial issue for fish of larger size (*see* Chapter 9).

Optimum food particle size for carnivorous fish has been suggested to be about a 1:1 ratio with mouth width for soft feeds and 0.5 of mouth width for hard pellets (Knights 1985). Optimum pellet size has been experimentally determined by Wankowski and Thorpe (1979) to be a factor of about 0.02-0.025 of fork length for Atlantic salmon fry and parr and about 0.01-0.09 for first feeding fry; Pacific salmon fry may utilize a slightly larger particle size. In further work on the effect of feed characteristics, Stradmeyer et al. (1988) have shown that Atlantic salmon parr prefer long thin pellets to round or fat ones and that soft texture is always preferred to hard. It is a simple matter to make pellets conforming to these preferences (*see* Chapter 10), and fish culturists have long been aware that fish prefer soft texture. To date, colour of hatchery feeds does not seem to be of great significance, although some studies have indicated that colour, or at least contrast, can influence fish preference (Jacobsen et al. 1987). Palatability can also be increased by the addition of various substances such as betaine which appear to have organoleptic properties (e.g., Mackie and Mitchell 1985). Taste and texture of feeds, and possibly other qualities influencing the attractiveness of formulated feeds, are usually of greatest importance when fish are close to satiation (Brett 1979). These characteristics of feed may be important in attaining growth targets, and they are also very important for feeding at low temperatures where appetite may be reduced.

Two serious and related problems which beset most hatcheries are damaged fins due to agonistic behaviour (attack resulting in fin nipping) and strongly unequal growth rates requiring grading. Wide size variation may contribute to stress levels, physical damage (*see* review by Abbott and Dill 1989) and, in the case of Atlantic salmon, in lower production of S1 smolts (*see* Metcalf et al. 1992 for review). The existence of dominant and subordinate fish within a hatchery tank population has been demonstrated for several species of salmonids [e.g., Atlantic salmon, Metcalfe et al. (1992); Thorpe (1991): rainbow trout (*Oncorhynchus mykiss*), Metcalf (1986): chum salmon, Olla et al. (1992): Arctic charr (*Salvelinus alpinus*), Jobling (1985), and probably coho salmon, Dill et al. (1981) and see the general discussion in Knights (1985)]. Although size itself does not cause dominant status (Thorpe et al. 1992), size differences usually soon develop such that dominant fish are larger than subordinate fish through unequal feed apportionment (e.g., *see* Fenderson et al. 1968; Abbott et al. 1985), and possibly stress effects on the subordinate fish (*see* discussion in Jobling 1985; Knights 1985). In a mixed tank of fish, stable linear dominance relationships are probably not possible. Rather, aggression is expressed as sporadic attacks by more dominant fish on any of those less dominant (*see* Jobling 1985). Presumably there is a genetic component to dominance and subordinance (Jobling and Reinsnes 1986). As noted above, the juveniles of certain species may be more prone to this behaviour, which may result from their life history patterns as wild fish. Some species of fish that are pelagic as juveniles (e.g., sockeye salmon) or throughout life [e.g., some strains of Arctic charr, lake trout (*S. namaycush*)] may show less aggression in captivity (Ferguson et al. 1983). This might be extrapolated from discussions in Noakes and Grant (1992) as an explanation for the Arctic charr's tolerance for very high density loading in culture relative to other species of salmonids (*see* section on density). It appears however that all salmonids are capable of some degree of agonistic behaviour under the right circumstances.

Increase in water velocity to reduce scope for aggression is discussed in the rearing container section (page 392) as a possible explanation for increases in growth and conversion efficiency noted at higher swimming speeds.

Fish culturists emphasize feeding as the major opportunity for dealing with the results of agonistic behaviour (or for incurring the negative results of this behaviour) and that certain feeding strategies may reduce agonistic behaviour and hence lead to fish populations with lower variance in size [*see* Huntingford and Thorpe (1992), and Noakes and Grant 1992 for general discussion, and Olla et al. (1992) for specific studies]. It is known that hungry fish tend to be more aggressive (Symons 1968), that damaging interactions often occur during or after feeding and that these are the cause of frayed fins and other damage (Abbott 1985; Klontz 1993). Piper et al. (1982) noted that fin damage tends to begin in small fish (ca 7.5 cm) and continue to about 25-27 cm. They determined that fin damage (and presumably agonistic behaviour) was markedly reduced in fish fed a high ration and also in fish starved over long periods. They concluded that very well fed fish were less aggressive during feeding and suffered less fin damage (*see also* Olla et al. 1992). There is also an interaction between aggression and fish density in tanks (*see* page 388).

In the hatchery another possible approach to reducing agonistic behaviour is to distribute feed evenly over the surface of the rearing container so that feed is difficult to defend (creating scramble competition - e.g., *see* Olla et al. 1992). This is usually done as a matter of course during hand feeding, but some automatic feeders deliver feed only to a small area of the tank. As noted in the section on rearing containers, Atlantic salmon are often fed in such a manner that feed comes to the fish, eliminating need to swim out of a territory area. This reduces aggressive incidents (*see* Needham 1988 and Drope 1990). This approach has been applied to rainbow trout (Klontz 1993), although it rests more on intuition and observation than on experimentation.

For smaller fish, increasing the number of meals per day, in addition to increasing the size of maximum ration, is thought to increase the opportunity for less dominant (subordinate) fish to achieve full ration. It is generally agreed by hatchery workers that smaller fish require more meals per day than larger fish, and that too few meals per day (at any ration) increases size variance (e.g., Leitritz and Lewis 1976; Piper et al. 1982; Fowler 1989). A single satiation meal very likely favours a higher share of food going to the more dominant fish in the population, which then become larger (and perhaps then more dominant). This is especially true if fish are fed to satiation too quickly, resulting in a "false" satiation (*see* Brett 1971*a* for discussion of time to satiation). Radiographic studies of feed intake allows study of differential feeding within a population of fish (Talbot and Higgins 1983). Abbott (1985) noted lower degrees of aggression as the between feeding interval was reduced in steelhead trout. Jobling (1983) determined that Arctic charr showed increased size variance on restricted diets with a long interval between meals. More frequent feedings (in this study, with higher daily ration) showed lower size variance, but the maximum meal frequency in Jobling's study was twice per day. Ruohonen (1986) found that 60 feedings per day maximized growth rates and minimized size variance in Atlantic salmon and concluded that dominant fish did not increase their growth rates but that the benefit of many feedings accrued to the less dominant fish.

Although offering fish several meals per day is widely felt to increase evenness of growth rate (reduce size variance) in populations, a steady introduction of feed throughout the day, as accomplished by some types of automatic feeders, is thought to favour the dominant fish by allowing territories to be set up. This would be especially true if the feed were introduced at a point source (Ryer and Olla 1991). One hatchery and feed lot practice is to feed to apparent satiation over 10-20 minutes. Then after a brief waiting period (perhaps while feeding another group of fish) to repeat feeding to satiation. The theory is that the second feeding is being done primarily by less dominant fish, while the dominant fish are hydrating their feed and showing reduced appetite. The effect of feeding strategy may also affect water quality, and some hatcheries reduce meal frequency to allow clearing fines from the water.

As noted earlier, small fish require more frequent feedings even though they have relatively larger stomach capacity. Because gut emptying time varies inversely with size, more frequent feeding may result in a higher maximum daily ration and usually higher growth rate. There is some information, however, to suggest that multiple feedings per day also result in a decrease in feed conversion efficiency, presumably due to lowered assimilation caused by forced food passage through the gut and incomplete digestion (Crampton et al. 1990). Similar conclusions may be inferred from studies with larger fish reported by Eriksson and Alanärä (1992). This is a serious issue for on-growing of larger salmon where feed costs are a very large factor in overall operations costs and profitability. In this situation reduction of conversion efficiency through over-feeding can be very costly. However, as noted, with respect to rearing of the smaller life stages, there are two major goals related to feeding strategy: The first is to reduce agonistic behaviour and resulting stress levels and injuries, and to minimize increases in population size variance due also to agonistic behaviour. The second is to choose a feeding frequency and ration level that optimizes growth rate and, to a much lesser extent, conversion efficiency. It may be that for larger fish, meal frequency should be restricted (e.g., *see* formula proposed by Crampton et al. 1990) and attention be paid to the mode of presentation. For example, one might allow ample time to satiation or offer a meal in two installments close to each other in time. Smaller fish may require different strategies.

Despite the work cited above, a need exists for more research on the optimum number of meals for fish of varying sizes and the effects of meal frequency on population size variance, maximum intake and growth rates. It is likely that optimum meal frequency will vary strongly with fish size and also with species and other aspects of the culture environment. There are many informal hatchery studies on feeding, often unpublished, and quite frequently these are designed without experimental rigor (e.g., *see* Rutherford 1992). These can, however, lead to hypotheses to be investigated more carefully through experiment. Rutherford (1992) provides a review of 25 years of hatchery trials related to feeding and diet in British Columbia salmon hatcheries; and represents a good example of this type of work, which is common throughout the world where salmonids are reared.

The importance of feeding at specific times of the day has yet to be well evaluated, although there are indications that it could be important (Kadri et al. 1991; Eriksson and Alanärä 1992). It is often noted during the on-growing of larger fish, for example chinook salmon, that fish feed better early in the morning and at dusk, although again this has not been quantified (but *see* Crampton et al. 1990). Such dawn and dusk rhythms are certainly noted in wild stream parr (above references and *see* Mundie and Mounce 1978; Mundie et al. 1990) and lake-rearing sockeye (Levy 1990). Although feeding rhythms vary with food availability, it is possible that these persist under different environmental conditions including those of the hatchery, although they certainly may be overridden by hatchery regime. If fish have innate rhythms of feeding, then perhaps various endocrine functions related to the processing of food at the gut level might operate at these times. This could lead to more efficiently processed ingested food (e.g., *see* review by Bell 1976). Similarly if fish have been trained to expect feed at certain times of the day, then they may develop similar biochemical patterns. Although this has not been well studied in fish, it is well known in other areas of animal husbandry.

Seasonal patterns of feeding as noted for wild fish are also well documented in hatcheries (*see* Piper et al. 1982). It is very common to exceed feed table recommendations in the spring and feed less than the tables suggest in the fall. This seems to be somewhat independent of temperature and probably is usually related to photoperiod (*see* Brett et al. 1969). These rhythms may be endogenous (for example *see* Jobling 1987; Eriksson and Alanärä 1992), and entrained by photoperiod under natural conditions. However, such patterns may not be apparent in domesticated strains of fish. In one hatchery trial, it was found that coho salmon fingerlings could be fed much less than the usual ration in winter and still catch up to fully fed controls in the spring before release, after being placed on maximum ration (Griffeon and Narver 1974). This suggests potential savings in feed and labour for some hatchery situations.

Compensatory growth phenomena are discussed in Chapter 7. There is an hypothesis that fasting periods alternating with intense feeding periods may provide equal growth on less food than constant feeding regimes. This is an area of relatively little study on fish that could have importance for feeding schedules. As new studies appear, it will be important to differentiate between the effect of gut emptying time and food intake, and genuine gains made by periods of fasting. For example at low temperatures it may be recommended large fish be fed every other day because stomach emptying time is longer than 24 h. A true compensatory effect may require several days of fasting followed by several days of feeding; a mechanism might involve differential use of lipid and protein or a constant growth of bone and connective tissue with intermittent protein deposition. Any feeding regime involving fasting periods will have to consider fish stress and aggression levels, species differences, and seasonal cycles.

### *Density*

Rate of flow and quality of incoming water are the first parameters to be dealt with in establishing suitable living conditions for fish. With high flow rates (or oxygen supplementation), another variable, the number or biomass of fish per unit volume or area, becomes limiting. This is known as *density* and may be expressed in several ways. It is the "elusive and controversial parameter" of fish culture (Westers 1987). When density becomes too high, fish are more prone to disease, show reduced growth, and have a poor appearance (Piper et al. 1982; Needham 1988) and their future performance (growth rate or survival) may be compromised, especially if they are to be released into natural waters (Wedemeyer 1976; Fagerlund et al. 1989; Banks 1994; and *see* page 419).

Reduced growth rate is often the first noticed of high density effects, and has been well documented (e.g., Brown 1957; Refstie 1977; Fagerlund et al. 1981; Fernø and Holm 1986; *see* Chapter 7). The mechanisms underlying growth reduction may be related to stress through changes in the pituitary-interrenal or pituitary-thyroid endocrine axes (Wedemeyer 1976; Pickering 1987; Leatherland and Cho 1985; Schreck et al. 1985) or the effect may be due to increased aggression or other behavioural interactions, which either limit food intake or reduce feed conversion (Keenleyside and Yamamoto 1962; Fenderson and Carpenter 1971; Refstie and Kittelsen 1976; Pickering and Stewart 1984; *see also* Chapter 7).

Aggression, itself a stress on some members of the population, may increase with density (Fernø and Holm 1986) and can cause loss of eyes and reduction or loss of fins (Banks and Fowler 1982) with consequent reduction in quality and ability to survive (Westers and Copeland 1973; Fagerlund et al. 1983; Banks 1994) although there may be other causes of these problems (for example, Lemm et al. 1988; Halver 1989).

Increased losses to disease have long been associated with high densities (for example, Haskell 1955; Wood 1979) and may be due in part to increased horizontal transfer of pathogens or to increased susceptibility due to the effects of stress on the immune system (Wedemeyer et al. 1990; Barton and Iwama 1991). It is often difficult to separate density effects from the often-associated deterioration of water quality (e.g., Poston and Williams 1988). Even when oxygen and ammonia levels are kept within safe limits, seston levels leading to gill diseases may increase with high densities (Wood 1979).

Research studies frequently fail to specify all of the significant conditions prevailing in a study or may be too specific too allow broad generalization. Consequently the mechanisms accounting for density effects are generally unclear. For example, light level, current velocity, disturbance, fish experience, feeding level and regime, and species may all affect tolerance to density; it is usual for several of these factors to be omitted from study descriptions.

Light intensity has been noted by fish culturists to affect performance and behaviour in fish at least partly through uneven distribution in tanks which may create aggression and stress. This may be related to use of cover by wild parr (e.g., Hartman 1963 and *see* discussion in Huntingford and Thorpe 1992), and varies with species (e.g., Pickering et al. 1987.) Atlantic salmon fry and parr are usually reared in low light intensity (ca 50 lux) because they move more readily into the water column and display less aggression (Needham 1988; Drope 1990). The effect of light must vary with the range of light intensities considered; light intensity has been shown to affect growth performance in density studies (Blackburn and Clarke 1990).

Current velocity may also influence behaviour in wild fish (Noakes and Grant 1986), and significantly affects performance in culture tanks (*see* review by Christiansen 1991 and discussion on page 400). Adjusting of current velocity and feed presentation strategy can reduce aggression and competition for food (e.g., Christiansen and Jobling 1990). Careful control of water velocity is used to rear Arctic charr at high density, and newer methods of rearing Atlantic salmon involve control of light intensity, velocity and feed presentation to induce full use of the water column (Babtie et al. 1986; Needham 1988).

Several investigators have suggested that there is an optimum density for some species above or below which performance decreases. This appears to be the case for Arctic charr (Wallace et al. 1988), in which a decrease in specific respiration rate with increased density was noted by Poston (1983). Aggression has been noted to increase and decrease as density is increased in Atlantic salmon (Keenleyside and Yamamoto 1962; Drope 1990). Tolerance to various density levels is affected by acclimation or previous density experience (Keenleyside and Yamamoto 1962; Fenderson and Carpenter 1971; Refstie 1977). Loftus (1983) noted that abrupt changes in density had a strong effect on growth performance of steelhead, and it is often noted in hatcheries that fish require considerable adjustment time after a tank or density change before effectively distributing themselves in the pond volume. In Atlantic salmon hatcheries, pond depth is often adjusted gradually to bring fish off the bottom and accustom them to maximum densities (Drope 1990). This is a relatively unexplored area in fish culture research.

The choice of fish density level will be determined by species reared, fish size, rearing container, water quality, culture methods, and other environmental parameters. Choice is often left to the intuition of the experienced fish culturist (Leitritz and Lewis 1976). Of major importance are the goals of the hatchery. Some loss of growth performance due to high density may be acceptable if total production per growing cycle is increased and the product has a good appearance (Trzebiatowski et al. 1981; Papoutsoglou 1987). If pathogen-free water is used, risk of increased disease losses may be less than if surface water containing pathogens is used in the hatchery (Wood 1979).

Some species, Arctic charr as noted, appear quite resistant to high density conditions (Wallace and Kolbeinshavn 1988), while others, such as Atlantic salmon may be more sensitive to high density levels (Gaston 1988).

The issue of smolt performance after release into natural waters deserves special mention. Several studies have indicated that ocean survival of hatchery smolts is inversely related to rearing densities in the hatchery (Fagerlund et al. 1983; Banks 1987; Sandercock 1987; Fagerlund et al. 1989; Banks 1994). The reasons for this phenomenon are not clear but could involve stress-induced physiological compromises including loss of hyperosmotic regulatory ability and resistance to pathogens (Schreck et al. 1985). The uncertainty associated with survival in natural waters after release (e.g., ocean survival of hatchery smolts) should indicate a conservative approach to the setting of density levels (*see* page 400).

Defining density is difficult, partly because the mechanisms by which density affects fish populations are not clearly known. The most usual expression of density is in fish weight unit per unit of water volume (which includes the displacement volume of fish) as kg/m$^3$ or g/l. This has the advantage of allowing comparisons between species, tank sizes, and sizes of fish. However fish are often unevenly distributed throughout the tank and actual densities may be very much higher than densities calculated on the basis of total water volume. Some species have been treated as essentially two dimensional (for example, Atlantic salmon in traditional literature: Gaston 1988), and in these cases density may be expressed as either weight or individuals per unit of area, usually kg or fish per m$^2$. It is also possible to combine numbers of fish and biomass in a single expression, for example, as in the density index of Piper et al. (1982).

Because fish metabolic rate and activity are inversly related to body size (Brett and Groves 1979), it is frequently assumed that smaller fish are more sensitive to density than larger fish (or that interference between fish at high densities will be more acute when there are more individuals per unit of volume or area) if biomass is held constant (Haskell 1955). To incorporate this relationship, Piper (1975; Piper et al. 1982) developed the *density index*. Based on hatchery experience, the index is a guide to safe rearing of fish at a density (in pounds per cubic foot) of one half their length in (or in kg per m$^3$, of three times their length in centimetres). Under this guideline a 12-cm salmonid would have a permissable rearing density of 36 kg/m$^3$. As a formula:

$$W = D \cdot V \cdot L \tag{7}$$

where W is the permissable weight (in pounds) of fish in a tank; D is the density index; V is the volume of the tank in cubic feet; L is average fish length in inches. The density index of 0.5 recommended by Piper et al. (1982) indicates a range of densities from about 14 kg/m$^3$ to about 50 kg/m$^3$ for 1-g and 50-g fish respectively. This would reflect a change in density expressed as individual fish per m$^3$ from about 14,000/m$^3$ to only about 1000/m$^3$. Density during rearing may fluctuate considerably depending upon the grading strategy, frequency of splitting of populations, and the availability of tanks of different sizes. Sometimes a reported maximum density occurs only for a brief time toward the end of a rearing period.

Although this very useful formula approximates the practices of many hatcheries, there are cases where it does not appear to apply owing to the goals of the hatchery, species reared, or other fish culture conditions that may ameliorate or intensify the effects of density. Some density studies have reported density loadings several times those usually chosen for production hatcheries. For examples, Buss et al. (1970) grew rainbow trout to densities of over 500 kg/m$^3$ in very small containers and densities of about 135 kg/m$^3$ in production silos with no apparent ill effects. Trzebiatowski et al. (1981) reared this species at densities from 32-150 kg/m$^3$ with success. Soderberg and Meade (1987) reared lake trout at several density ranges including 221-245 kg/m$^3$ over a 97-day period with no apparent growth reduction, increase in mortality or increased fin erosion compared to controls. The high density corresponded to a density index of 2, four times higher than the recommended 0.5 (above). In a similar study Poston (1983) reared the same species to densities of 230 kg/m$^3$ with little apparent ill effect.

The lake trout has been shown to be a less territorial fish than many other salmonids (Ferguson et al. 1983), but in a similar study, Soderberg and Meade (1987) reared Atlantic salmon, generally considered a territorial and aggressive fish, at densities to about 90 kg/m$^3$, which is 2-3 times greater than that usually seen in commercial smolt hatcheries. Wallace et al. (1988) reared Arctic charr to densities of 170 kg/m$^3$ with no growth or condition changes relative to controls, and larger Atlantic salmon have been reared in salt water at 100-125 kg/m$^3$ with no significant performance reduction compared to controls (Kjartansson et al. 1988). There also have been studies of duo- and polyculture where two or more species of salmonids are reared together, sometimes with a greater permissable density than either species alone (Holm 1989; Nortvedt and Holm 1991; Holm 1992).

Despite these and other trials at very high densities, most commercial (private) and enhancement (government) operations prefer to rear salmonid fry at densities from 15 to 30 kg/m$^3$ and note growth reductions and fin damage at higher densities. Pacific salmon are reared at densities below 15 kg/m$^3$ in government hatcheries (Sheperd 1984; Sandercock 1987). Accelerated coho salmon smolts have been reared successfully at densities of 25 kg/m$^3$ (Blackburn and Clarke 1990), and commercial hatcheries rear the same species and Atlantic salmon at about 30 kg/m$^3$. In France, by contrast, Atlantic salmon densities are kept as low as 7 kg/m$^3$ (Gaignon 1987). Densities of over 30 kg/m$^3$ for this species may reduce smolt quality (Babtie et al. 1986; Needham 1988). Rainbow trout are reared at somewhat over 25 kg/m$^3$ in many countries (Ingram 1988).

The reasons for the apparent discrepancy between published density studies and hatchery practices may be related to tank size; most of the high density studies have used fish containers of a smaller size than those used for production. As tanks or raceways become larger, it is more difficult to control flow and flushing dynamics (*see* below). Also the time allowed for care and feeding may be reduced in a commercial or production setting. The element of risk should also be considered; fish held at higher densities may be subject to higher risk of disease outbreaks and very high densities result in short response times to asphyxiation in the event of water flow or oxygenation failure. Nonetheless with more information on the effects of environmental variables on fish behaviour and with well designed tanks, it should be possible to increase rearing density beyond that most currently practiced without jeopardizing fish health and hatchery production.

### *Characteristics of Fish Rearing Containers*

*Introduction.* There are many types of fish rearing containers in use, each with a spectrum of advantages and disadvantages. They are chosen for reasons of cost, utilization of space and various fish rearing considerations, but frequently experience and predilection of the fish culturist are major factors in choice of container. Major fish culture variables include water velocity and velocity distribution, sedimentation and cleaning, and flushing or water exchange. Velocity control is important for exercising fish and controlling fish behaviour, including aggression (*see* page 400) and distribution, and to clean the tank bottom. Flushing characteristics include the distribution of water quality parameters throughout the tank such as oxygen, ammonia, and suspended particulates, which in turn may influence fish distribution. The best fish rearing tank would have several qualities, usually not all attained in one tank design: (1) the velocity could be set to an optimum throughout the tank so that all water would be exchanged with each replacement flow (when inflow becomes equal to tank volume); (2) the tank should be completely self cleaning; and (3) no variation of water quality should occur anywhere in the tank. It should be possible to control light level in the container. In most container designs, some of the above qualities, but usually not all, are achieved or approximated. Technical aspects of rearing container design, cost, and operation have been reviewed by Senn et al. (1984).

Two major types of containers are in use: circulating tanks and flow-through tanks. Circulating tanks are usually round or oval in shape and act as mixed-flow reactors in which new water entering the tank quickly becomes mixed with the resident water, then exits through a central drain. In an ideal mixed-flow reactor, flushing is not complete when inflow equals tank volume; only 63.2% of the water will have been exchanged at this time (Tvinnereim and Skybakmoen 1989). For example, if a 100-l tank of water has an ammonia concentration of 1 mg/l and a flow of 10 l/min ammonia-free water is begun at time 0, then after 10 min 100 l will have entered and left the tank, but the ammonia concentration will be 33.8 mg/l, not 0 mg/l. In so far as the ideal circulating tank approximates a mixed-flow reactor; water quality parameters and velocity will be homogeneous throughout the tank. Fish also will be distributed evenly throughout the tank and velocity will be high enough to keep the tank bottom clean.

Flow-through tanks include troughs and raceways and are typically long and narrow with water entering at one end and exiting from the other. They approximate plug (or slug) flow, that is, a bolus of water entering the upstream end of the trough or raceway flows through the vessel intact with little mixing. With perfect plug flow, the tank will be completely flushed (all water exchanged) when the inflow equals tank volume; there will be water quality gradients in the tank from inflow to outflow.

Idealized flow patterns are not attained in either type of tank; circular tanks exhibit short circuiting (some water goes quickly to the outlet without mixing) resulting in stagnant areas where replenishment by new water is retarded. Flow-through tanks never show complete plug flow; instead water mixes turbulently with resultant lags and short-circuiting.

It should be noted that the term "flow-through" and the term "single-pass" may be confused. Single-pass systems denote that water discharge rate is equal to water inflow rate. That is, no water is re-used after it leaves the fish tank. Both circulating and flow-through tanks may be single-pass systems.

*Flow-through Tanks*. Raceways and troughs appear to be the most common tanks used in the early years of salmonid culture. In these early days, young salmonids were typically reared in small wooden troughs until a few grams in weight, then transferred to earthen raceways. The similarity of these containers to natural streams was one reason for their use (Davis 1946), another probably was their low cost. Many fish culturists believe that the flow-through type of container is superior due to its plug flow characteristics; this is thought to clear metabolites from the container quickly and deliver clean water to fish, unmixed with used water. The smaller units provide very convenient access to fish, and it is easy to crowd or remove fish in them.

The actual hydraulic characteristics of raceways and troughs are complex (Burrows and Chenoweth 1955; Wheaton 1977; Vizcarra 1985). Friction of the container sides creates velocity gradients depending on distance from the sides and the bottom of the container and this creates turbulence and irregular mixing. Velocity is usually low in troughs and raceways, which may promote unstable flow regimes. The irregularities in velocity lead to mixing, especially in large or long raceways so that much of the plug flow attribute is lost. There is, however, enough direct flushing to make bath or flush disease treatments safe and convenient in smaller units.

The low velocities of troughs and raceways are generally below the settling velocity of fish feces and fish feed fines (about 3.3-3.5 cm/sec, Westers and Pratt 1977; Westers 1987; Cripps 1993; and *see* page 419) which therefore accumulate along the bottom. To be self-cleaning, velocities along the bottom should be greater than 6 cm/sec (Burrows and Chenoweth 1955), but in the flow-through container, velocity is controlled by cross-sectional area and inflow:

Velocity = Flow/Area (8)

Under most conditions of fish density and flow loading, velocities are very low and daily cleaning is required. Pond cleaning may be stressful to fish and is labour intensive (Wood 1979). This is a major disadvantage of flow-through containers although some believe that seston on the bottom is safer than suspended seston. There are various designs to make such tanks self-cleaning. Baffles may be installed at intervals down the length of the trough or raceway (Westers 1981; FRED 1983) with a gap between baffle and bottom where velocity is increased enough to scour detritus from the bottom. These baffles may be cumbersome but they are effective. A moving baffle has been designed which can be placed at the trough head once or twice daily for cleaning (Abraham et al. 1985), and there have been several designs for special sumps and screens which may facilitate solids removal (e.g., Wheaton 1977). Conversions introducing circulating design into flow-through tanks are also used (Burrows and Chenoweth 1955 - the Foster-Lucas Pond; Watten and Beck 1987 - the cross-flow rearing unit).

Distribution of fish in flow-through containers may be very uneven due to gradients in water quality (Peel 1983; Piper et al. 1982) or to variations in light intensity or disturbance, or orientation to current, and this may be a major disadvantage with such units. True density in the tank may be very much higher than calculated density due to local concentrations of fish.

*Types of Flow-through Rearing Units.* There is a continuum in pond types from small troughs to large open channels, but little firm agreement in terminology. Many containers informally bear the name of their designers, often without benefit of publication. Colloquial usage is followed here.

*1) Troughs.* Troughs are small rearing units ranging from 30 to 100 cm in width, 20 to 70 cm deep and 3-5 m in length (for representative designs, *see* Piper et al. 1982; Sheperd 1984). They were derived in North America from the Atkins incubation-rearing box (Atkins 1878). They are most often used for start feeding and rearing of very small fry, but should not be used for larger fingerlings (e.g., Johnson and Gastineau 1952). They are excellent for first feedings and facilitate observation, crowding, and other handling, and they are easy to move.

*2) Raceways.* Raceways (Figure 5) are larger versions of troughs, from 1-2 m wide, 0.3 to 1 m deep and 5-10 m long. Preferred proportions are, 1:3:10-30 for depth:width:length, (Piper et al. 1982; Westers 1987; and *see* discussion below), but many other proportions exist. Raceways may be free-standing, but are very often permanent structures partially or entirely built into the ground. As raceways become larger and longer, mixed flow becomes more predominant than plug flow. In the smaller raceways (and larger troughs), entry of the water must be symmetrical or serious dead areas form (Burrows and Chenoweth 1955). A baffle behind the inflow helps to overcome this problem; further baffles along the raceway create zones of higher velocities which, as noted, make the raceways self cleaning (Westers and Pratt 1977; FRED 1983.) The effect of baffles on plug flow characteristics has not been well evaluated.

Larger raceways pose some management problems. Cleaning and fish sampling are more difficult, and it may become challenging to maintain accurate fish inventory, which in turn can lead to overfeeding and increased cleaning needs. Well-designed concrete raceways do allow mechanical crowding and grading of fish, and automatic feeders can be spaced along the raceway to encourage dispersion of fish (Leitritz and Lewis 1976). Because of the somewhat mixed flow conditions, chemical treatments should be done with care.

*3) Earthen channels.* Rearing channels dug into the ground and lined with earth or crushed stone are an extension of the raceway concept, and are one of the oldest intensive fish culture designs. They can be very large — several metres wide and 20-100 m long with periodic dam structures to regulate depth and flow; they may be curved to conform to topography. The main benefit of such channels is the low cost of construction. The nature of the substrate makes cleaning impractical and biological activity maintains bottom quality; this requires relatively low loading density. Inventory is difficult to control. Both predation and losses through disease may go undetected and feeding rates must be gauged somewhat by feeding response to avoid bottom deposits of uneaten feed (Peel 1983). Demand feeders are sometimes used (e.g., Tipping et al. 1986). These usually improve feed:flesh conversion ratios, a sign that inventory was probably overestimated.

Long earthen channels or channels constructed in series to intensify water use may pose a significant risk of disease epizootics, which may be difficult to control. Initial water quality (e.g., temperature, pathogen load) is very important in reducing this risk. Proponents of earthen channels often feel that natural food sources supplement the artificial diets with resulting increases in fish quality (e.g., Davis 1946). Although this may be so, there is little formal evidence to support this belief. A modified earthen channel designed specifically to increase natural food supply has been designed and tested with some success (Mundie and Mounce 1978). This experimental channel consisted of alternating pools and riffles with enhanced stream insect production, supplemented by artificial feeds, with abundant overhead cover, both natural and artificial. Smolts were produced successfully using this method, although ocean survivals from the semi-natural channel releases were similar to but not higher than conventional hatchery releases from the same location. Mundie et al. (1990) discussed these results and concluded that such channels may be valuable for small scale enhancement projects.

*4) Earthen ponds.* Large irregular, shallow bodies of water (ca 0.1 - 3 ha) are frequently used to rear fingerlings and smolts. These possess all of the disadvantages mentioned above for earthen channels and allow even less control over the rearing process. Their main virtue is low cost of construction and operation. They are usually

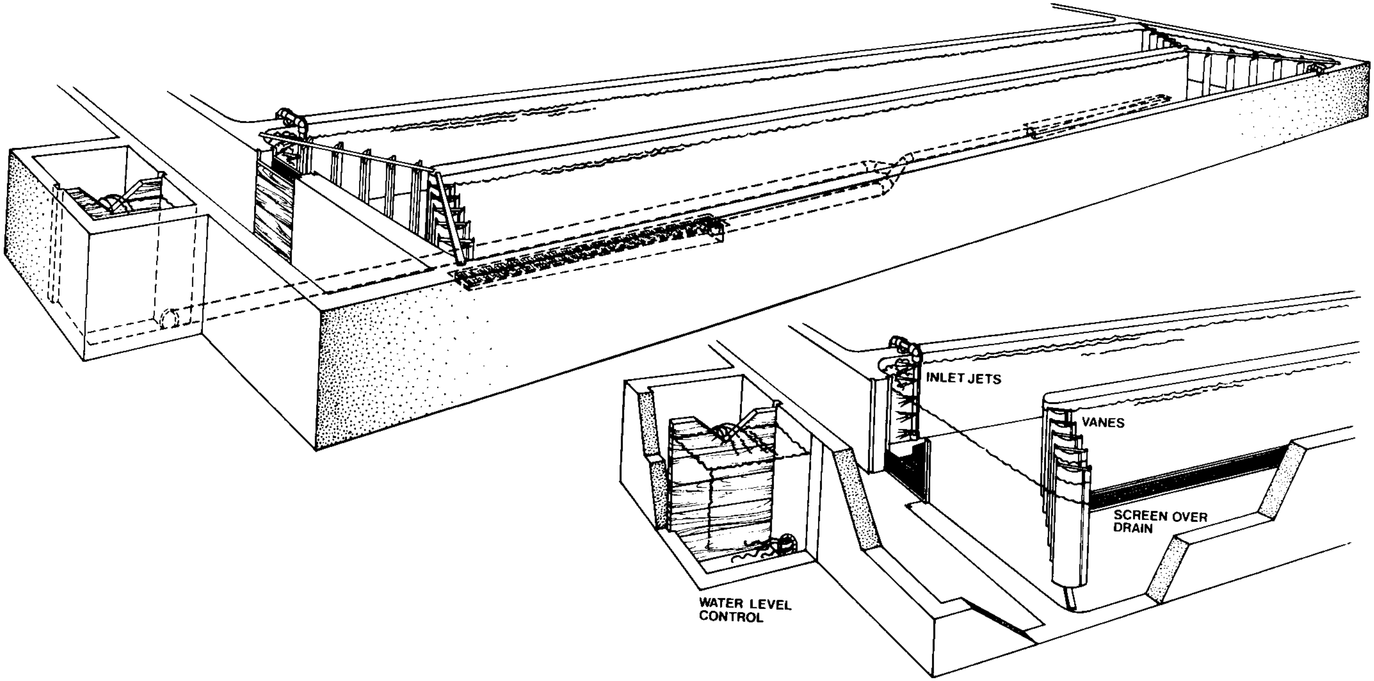

FIGURE 5. Raceways. Courtesy of the Canadian Department of Fisheries and Oceans.

stocked at a relatively low density to avoid problems with algal blooms and anaerobic bottom sediments.

*Circulating Tanks.* Although circulating tanks have been used for some time (e.g., Cobb and Titcomb 1930), they have been less popular than flow-through tanks until recent years. The most common type of circulating container is the round tank available in many sizes and proportions. Water inflow and outflow systems are also variable. Figures 6 and 7 show several common arrangements of round tanks. The relatively few rigorous studies of water circulation within such tanks (Burrows and Chenoweth 1955; Larmoyeux et al. 1973; Rosenthal et al. 1982; Burley and Klapsis 1985; Vizcarra 1985; Tvinnereim and Skybakmoen 1989) have shown that there is considerable departure from the ideal of a completely mixed-flow reactor as described on page 392. These studies (usually done in tanks without fish) often showed short-circuiting and the formation of dead volumes (areas where exchange is relatively reduced). Velocity usually varied throughout the tanks. The chief variables affecting exchange were tank proportions (depth to diameter ratios) and inflow and outflow design.

Single point inlets near the tank wall tend to create a central area of reduced exchange which may be torus-shaped with areas of more rapid exchange (and therefore short circuiting) along the sides. In this situation, the tank may be self-cleaning and the torus may act as a settling area for seston, which is then swept along the bottom to the central outlet (Burrows and Chenoweth 1955). For low loading densities this arrangement may be very satisfactory; fish have some choice of velocities and conditions will be adequate throughout the tank volume. At higher densities, the zones of reduced circulation will become marginal in quality and fish will not remain spread out evenly, but will congregate in smaller areas, further distorting the uneven water quality (Rosenthal and Murray 1986). Responses of fish to light or current may also cause similar problems. If fish are evenly distributed, their swimming activity and water displacement will increase mixing and reduce turn-over time.

Horizontal spray bars mounted radially over the tank surface serve two functions: First they appear to create much superior mixing and even distribution of velocities (Larmoyeux et al. 1973; Tvinnereim and Skybakmoen 1989). Second, the turbulence of the water jets very significantly aerates the tank water allowing higher flow loading levels than would be possible in submerged inlet tanks or raceways. The use of spray bars in circular tanks provides a significant advantage over other types of water inlets and flow-through type containers.

Diameter-depth ratios strongly affect flow patterns in tanks. The formation of central dead spaces is more pronounced in shallow tanks (depth:diameter ratios of 0.2 or less), and in large shallow tanks this may become serious (Larmoyeux et al. 1973). Tvinnereim and Skybakmoen (1989) tested point source inlets, vertical slotted manifold inlets, and spray-bars, and found that the point source inlet contributed to very poor mixing and that spray bar inlets produced the best mixing and most even distribution of

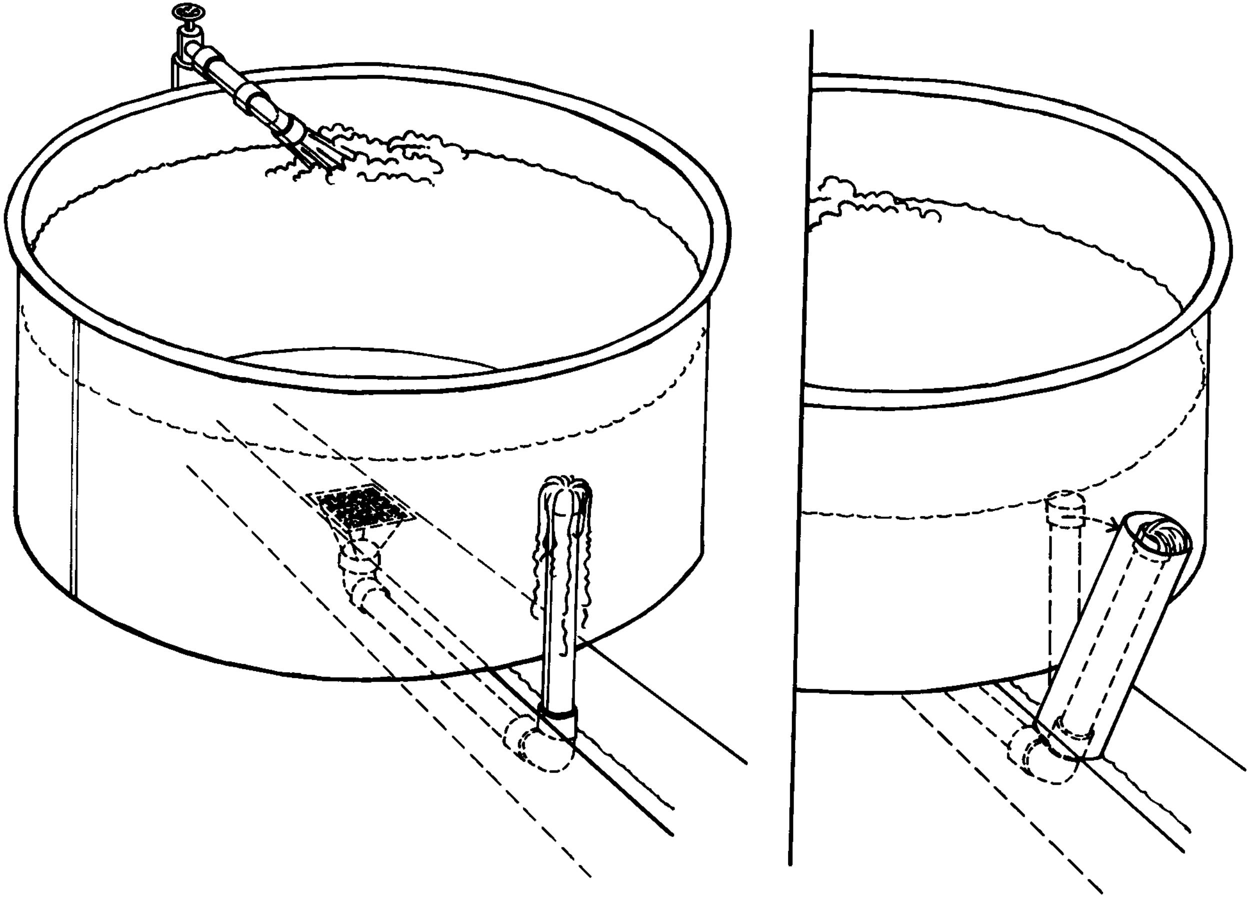

FIGURE 6. Courtesy of the Canadian Department of Fisheries and Oceans.

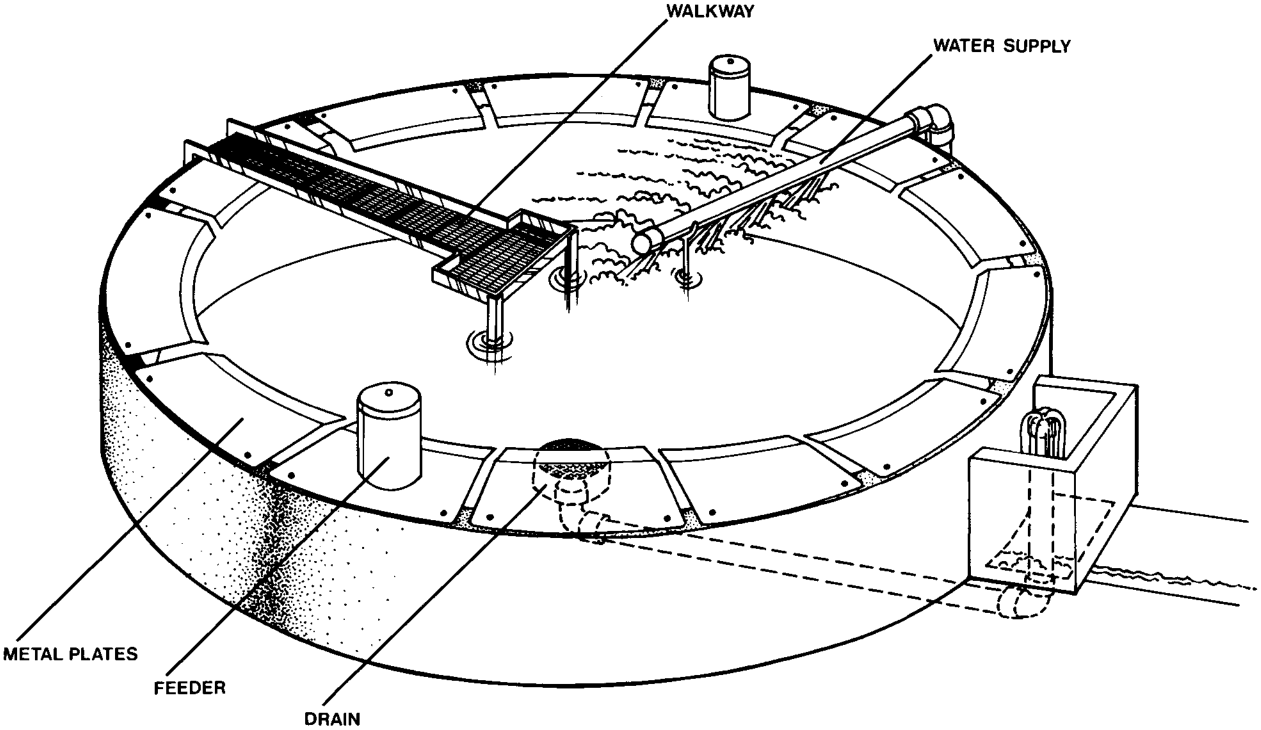

FIGURE 7. Courtesy of the Canadian Department of Fisheries and Oceans.

velocities. The vertical inlet was intermediate in its effects, and performed poorly in shallow tanks. Results of tests made on medium sized tanks (ca 2 m in diameter) may not be applicable to larger tanks unless a spray bar is used as the inlet. Very large shallow tanks may benefit from two spray bars at opposite sides of the tank.

Outlet designs vary (Surber 1933; Davis 1946; Larmoyeux et al. 1973; Burley and Klapsis 1985). Internal standpipes are effective when combined with outer screen cowlings. Central screens with external standpipes are more convenient for large tanks.

Velocity control is achieved with spray bars through the force of water (horizontal vector) from jets striking the water surface. The angle of the jets creates vertical mixing (turbulence) and horizontal movement (velocity), and by altering jet angle and water pressure the distribution of water quality parameters and velocities can be controlled. At very low flows, velocity may become too low for self-cleaning, and there may be a build-up of organic solids and fungi in the sumps or standpipes. Details of jet orifice and manifold diameters are given by Burley and Klapsis (1985). This and other literature cited should be consulted before setting up new tanks, since improper arrangements of inlets and outlets may severely compromise tank performance (Rosenthal and Murray 1986).

Because velocity may be controlled over such a wide range, it becomes important to determine the optimum velocity for fish, but this is complex. Work done by Brett and co-workers (Brett 1964, 1965, 1967, 1973; Brett and Glass 1973; Brett et al. 1958) clearly showed the relationships between swimming speeds and endurance of salmonid fish and the variables, fish size and temperature. From this work the range of fish swimming performance is well understood, but the application of this knowledge to practical fish culture is much less clear. This is now becoming the focus of new research.

It is sensible to suspect that fish to be released should be exercised first (Burrows 1969; Poston et al. 1969) and that exercise might lead to healthier fish and increased wild survival (e.g., increased returns to a hatchery or target fishery). Brett et al. (1958) showed that exercised coho salmon fingerlings developed greater endurance and cruising speeds than non-exercised fish. However relatively little work has been done to conclusively verify beneficial effects of exercise for fish to be released. Experiments on this topic would be difficult to design without confounding factors, (for example, changes in fish size and feeding due to exercise, which could affect density during rearing and subsequent survival in the wild).

Recently however considerable work has been done on the effects of rearing fish at different water velocities (exercise levels) on growth and physiology. Velocity control, for example as seen in circular tanks, allows exercising of fish which may be beneficial in terms of growth (Leon 1986; Houlihan and Laurent 1987; Totland et al. 1987; Barratt and McKeown 1988; Christiansen et al. 1989, 1991, 1992; Christiansen and Jobling 1990; and *see* review by Christiansen 1991). In summary, exercised fish appear to grow faster, usually with improved feed conversion ratios, than non-exercised controls. This work has focussed on beneficial effects of exercise on fish production in captivity, but not on survival of fish after release into wild situations.

The results of these and other studies are significant in relation to hatchery production and fish quality, although much remains to be learned. For example Totland et al. (1987) found that 2-kg Atlantic salmon placed in exercise tanks for 8 months grew 38% larger than sea-cage controls. The exercised fish had hypertrophied white muscle cells, a higher degree of aerobic capacity in the white muscle, and fewer signs of aggression (damaged fins). Conversion efficiency was slightly lower in the exercised fish.

Christiansen (1991) reviewed similar results for Arctic charr and suggested various explanations for improved performance seen. A marked reduction in aggressive behaviour was apparently due to more rapid swimming, which may have reduced metabolic costs incurred by chasing and attacking, and reduced stress levels which could also reduce respiratory rates. This could as well have led to better feeding of sub-dominant fish. Fish may also gain energy through ram ventilation thereby reducing opercular energy expenditures. The endocrine system may have been involved as some studies have shown anabolic hormone increases associated with exercise (e.g., Barret and McKeown 1988) and may explain the increased appetite and food intake that was seen. Hypertrophy of the main muscle mass (and greater weight gain from protein deposition), was noted in these studies. Physiology of teleost fish muscle is reviewed by Bone (1978) and with special reference to hatchery fish, by Johnston (1982).

More work must be done to quantify the beneficial effects of exercise on the species already studied and on other species not yet studied. Swimming speed per unit effort changes with fish length and with temperature (Brett et al. 1958; Fry and Cox 1970; Brett and Glass 1973) so that to obtain the benefits discussed above, an optimum swimming speed (water velocity) may need to be determined for fish of different sizes swimming at different temperatures. More work on the causes of increased performance is also necessary, and the possible effects on the ocean or freshwater survival of fish released into natural waters is of great interest. Practical hatchery protocols will need developing if these encouraging results prove to be general, and this will involve the control and measurement of velocities in fish tanks, fish behaviour and distribution in tanks, and possibly changes in tank design.

In conclusion, circulating tanks offer several advantages over flow-through tanks. Fish tend to be distributed evenly around the tank (Larmoyeux et al. 1973), and velocity can be controlled quite independently of flow rate. Exercise may improve fitness after release although this has never been conclusively demonstrated, but exercise does appear to have significant effects on fish growth performance. The additional aeration caused by spray bars allows greater loading rates and better utilization of water. Finally, the surface to volume ratio of circular tanks in comparison to raceways facilitates control of illumination, which itself improves eveness of fish distribution and facilitates certain photoperiod control protocols.

Critics of the circulating tank point out that water quality parameters affected by fish metabolism will equal those at the outlet of a flow-through tank, that is, will have the tank's poorest conditions throughout, rather than only at the outlet. This is because water is very well mixed in such tanks; water quality anywhere in the tank is similar to that at the outlet, and water quality at the outlet of any tank is the result of fish respiration throughout the tank (e.g., *see* Westers and Pratt 1977).

*1) Types of Circulating Tanks.* Deep round tanks (2-4 m diameter, 1-2 m depth) as discussed in the previous section are the most commonly used circulating tanks. Variations in proportions also exist and very large tanks have been used. Smaller tanks have the virtue of being moveable and, as noted, are easier to control. There are several drain designs, some including exterior dead fish collecting boxes. Some tanks have bottoms which slope to the centre, but this is probably unnecessary for proper self cleaning (Larmoyeux et al. 1973).

The Burrows Pond (Burrows and Chenoweth 1955) is a modified raceway with circular flow around a longitudinal central septum and two floor drains. It acts as a circulating pond while still retaining some characteristics of the raceway. This system is used with success in many hatcheries in the Pacific Northwest.

Other tank designs include the Swedish square circulating tank with rounded corners, used in many indoor hatcheries to conserve space. The corner areas may produce turbulence and interfere with mixing and self cleaning (Klapsis and Burley 1984) but with modified water inlets and outlet screen design, it can be made to perform as well as a round tank (Burley and Klapsis 1985). The 21% space saving will be of benefit only if the tank performs properly.

A special rearing "radial flow" tank has been designed by Thorpe (1981) to accommodate Atlantic salmon by reducing aggression and making feeding opportunities equal all fish. This ingenious tank is useful for research studies or production of small lots of salmon smolts. Floating vinyl cones have been used by coastal hatcheries to rear fry and fingerlings (FRED 1983). The cones are surrounded by a floatation collar which may also serve as a walkway. Water enters at the surface by the usual inlet pipes and exits through a screen in the bottom directly into the marine environment. Hydraulic head maintains the shape of the cones; salt water can be introduced by venturi action through the freshwater inlet pipe for acclimating smolts. Where flat land is limiting, such systems offer expansion which might otherwise be impossible.

Silos are very deep circular tanks with various inlet and outlet structures (e.g., Buss et al. 1970), which maximize use of space, but which may have complex circulation patterns (Rosenthal and Murray 1986).

### *Materials Used to Manufacture Fish Tanks*

Fish tanks can be made with any waterproof material strong enough to maintain their shape when filled. Other key characteristics include smooth surfaces, resistance to ultraviolet light and very low temperatures, ease of repair, and low cost. There should be no toxic leachates in the material. If metal containers are used the correct alloy must be chosen for waters of low pH or high salinity in order to resist corrosion. Moreover, ions of some metals in solution can be quite toxic to fish (e.g., copper, zinc). Aluminum is often used for troughs although it is expensive. Fibreglass (glass reinforced plastic) is the most common material for small to medium-sized circular tanks and troughs. Fibreglass tanks are moderately expensive, but when well made last a long time, have a very smooth surface, and hold their shape. Polyethylene tanks are available in many countries and are less expensive than Fibreglass or aluminum, but they do not retain their shape as well and are subject to scratches and abrasions (Wheaton 1977).

Large tanks may be made with concrete, which requires a smoothing inner coating. These are very cost effective, but retro-fitting or removal may be difficult or expensive as they are very permanent structures. In some countries asbestos-cement tanks have proven effective and inexpensive. Temporary tanks can be made with wooden or metal frames and vinyl liners.

## WATER FLOW REQUIREMENTS

### *Introduction*

Degradation of water quality occurs whenever there are large numbers of fish in a limited water supply. When fish respire, oxygen is consumed and carbon dioxide and ammonia are produced. There is also production of fecal solids if fish are actively feeding. If the water supply cannot flush away wastes and replenish oxygen the quality of the environment is degraded. This can occur during egg/alevin incubation, juvenile rearing or adult holding. It is a common concern in fish culture but can also pose problems for wild fish.

For example, Foerster (1968) reported the loss of 80,000 wild adult pink salmon migrating to a stream in South East Alaska. Low flow and high fish density resulted in low dissolved oxygen and high carbon dioxide concentration, which resulted in mass suffocation. Similar incidents have occurred on the Glendale River in B.C. (Seppala 1990).

In the fish culture setting, degradation of water quality due to high fish density is a central issue in the design and operation of facilities. Water supply is a major expense in culturing fish and so the loading rate Lr, or ratio of biomass B (kg) to flow Q (l/min), usually is high. This results in significant changes in ammonia, carbon dioxide, suspended solids, and dissolved oxygen as water passes through the facility. The optimum loading rate depends on many factors such as temperature, fish size, pond design and background or initial water quality. It also depends on the program objectives. Optimum loads may be lower where smolt quality is of prime importance — e.g., in ocean ranching where adult returns (rather than biomass produced in the pond) is the criterion for success.

The carrying capacity of a water supply can be increased by treating and re-using the effluent or by oxygen supplementation of the inflow water. This is particularly important if water flow is limited or if rearing water has been heated to accelerate growth. Re-use can greatly reduce heating costs. Reconditioning fish culture effluent by removing suspended solids, ammonia, pathogens and carbon dioxide and by adding dissolved oxygen is complex and expensive. In theory, treatment of the effluent followed by re-use could increase the carrying capacity to very high levels. Increasing the dissolved oxygen of the inflow water (oxygen supplementation) is a less expensive way of increasing carrying capacity. However only modest increases can be achieved. Oxygen is added because it is often the first limiting factor and also because it can alleviate the effects of metabolites and high temperature.

### *Water Flow Requirements for Single-pass Systems*

*Prediction of Limiting Factors and Carrying Capacity.* Colt and Orwicz (1991*b*) have developed a broad framework for determining water flow requirements for aquaculture. Depending on background, water quality, oxygen, ammonia, carbon dioxide, suspended solids and pH can all be limiting. For example if background pH is high, ammonia can be the first limiting factor. This occurs because the un-ionized (toxic) fraction increases rapidly with pH. If the water supply has an extremely low alkalinity (buffering capacity), pH can be limiting. Without natural buffers, carbon dioxide produced in respiration lowers pH dramatically. In this case the maximum loading rate Lr would be determined by pH at the outflow of the pond. Suspended solids concentration (waste feed and feces) can also become limiting before oxygen depletion. During early rearing, fry are often exposed to high levels of suspended solids (waste food and feces) which can cause chronic gill irritation even though oxygen requirements are met (*see* page 380). However, in the majority of cases where background water quality is normal and there is little gas exchange with the environment, dissolved oxygen becomes the first limiting factor as biomass of fish increases.

*1) Dissolved oxygen depletion.* In a raceway type pond, where fluid moves in an orderly fashion from inflow to outflow, the oxygen concentration decreases down the length of the raceway from Ci at the inflow to Cf at the outflow. In circulating ponds where the inflow water is uniformly mixed almost immediately, the oxygen concentration is the same throughout the pond and there is no gradient. In this case the oxygen concentration throughout the pond is given by the outflow oxygen Cf.

In either type of pond Cf (mg/l) is related to the oxygen consumption rate of the fish Ro (mg of oxygen consumed per kg of biomass per h), load rate Lr (kg of biomass per l/min of water flow) and Ci (mg/l), and is predicted by Equation 9:

$$\mathrm{Cf} = \mathrm{Ci} - \mathrm{Ro}\ \mathrm{Lr}/60 \tag{9}$$

This relationship is only true under steady state conditions (i.e., when Ro and Ci are constant). When Ro or Ci vary with time the response at the pond outflow will lag the value predicted by Equation 9. The lag time depends on the mean residence time (volume/flow) of the pond. For ponds where the mean residence time is under 90 min, Equation 9 can be used to predict the average daily and minimum daily Cf value with negligible error (McLean et al. 1993*b*).

To predict the carrying capacity of a water supply when oxygen is limiting, Equation 9 can be rearranged to give Lr:

$$\mathrm{Lr} = (\mathrm{Ci} - \mathrm{Cf})\ 60/\mathrm{Ro} \tag{10}$$

If Cf is set at a minimum acceptable level then Lr is maximized and predicts the carrying capacity of the water supply. The maximum biomass B (kg) that can be carried for a given flow Q (l/min) is given by Q∗Lr. Realistic values for Ci and Ro must be substituted into Equation 10 to determine carrying capacity.

> *a) Initial DO concentration, Ci.* The initial dissolved oxygen (DO) concentration, Ci, depends on the solubility of oxygen and the degree of saturation of the incoming water. Oxygen solubility decreases with temperature and salinity and increases with barometric pressure (Hitchman 1978; Colt 1984) (Figure 8A). Fresh water at 10°C at sea level (BP = 760 mmHg) has an oxygen concentration of 11.3 mg/l at equilibrium with the atmosphere (100% saturation). Most fish culture facilities have aeration systems that increase oxygen to at least 95% of saturation; Ci in this case is 0.95∗11.3 or 10.7 mg/l. If oxygen is supersaturated to 150% of saturation, Ci is 1.5∗11.3 = 17.0 mg/l.
>
> *b) Oxygen consumption rate, Ro.* The oxygen consumption rate of the fish Ro is a complex dynamic variable that is difficult to model. It depends on temperature, fish size, feed rate, ration quality, species, and activity level and can vary rapidly over time. Ro variation over a 24-h period is shown for chinook salmon juveniles at Nitinat Hatchery on Vancouver Island (Figure 8B). Dissolved oxygen was monitored continuously at the inflow and outflow of a raceway containing 586,000 4-g fish. Ro plots are shown for May 5 and May 17, 1990. The average daily temperature (11.3 vs 10.7°C) and ration level (2.85 vs 1.67% dry/d) were higher on May 5. This caused an increase in Ro during the late afternoon and evening of May 5 (Figure 8B).

Many models have been developed to predict Ro. Westers (1981) recognized that obtaining and processing food is a prime determinant of oxygen consumption rate and reported that 200 g of oxygen are consumed by salmonids for every kilogram of food consumed.

From and Rasmussen (1984) developed a theoretical model for rainbow trout that relates average daily oxygen consumption rate Ro to fish weight, temperature and ration level. In this model ration level is expressed as a fraction of the maximum ration.

Muller-Feuga et al. (1978) derived a model for Ro that is sensitive to temperature and fish weight. This model assumed that the maximum ration level was being fed as forecast by feed manufacturer's charts. The Ro values forecast by this model reflected the oxygen consumption rates associated only with digestion. Oxygen measurements which were started 5 or 6 h after the last food intake were performed every 3 min over a 1-h interval. Thus, the oxygen consumption associated with the excitement of feeding was not included.

Liao's (1971) oxygen consumption rate model assumes standard hatchery feeding rates, is sensitive to temperature T and fish weight W, and has the form $Ro = K\ T^n\ W^m$. Fivelstad and Smith (1991) used the same equation to model Ro data for Atlantic salmon post smolts (200 to 850 g) in seawater between 5 and 9°C. Elliot (1969) also developed a predictive model that was sensitive to temperature and fish weight and hence assumed a standard hatchery feeding level.

Colt and Orwicz (1991*a*) related oxygen consumption rate to feed rate. The average daily oxygen demand or mass of oxygen consumed per day was proportional to the daily ration. The proportionality constant or oxygen to feed ratio (OFR), decreased with ration size according to: $OFR = 0.621\ F^{-0.8}$. Converting to common units of mg of oxygen consumed per kg of fish per hour, the average daily oxygen consumption rate is given by: $258.8\ F^{0.2}$ where F is the feed rate in units of % body weight per day (%/d or g of food per 100 g of fish per day).

McLean et al. (1993*b*) related Ro to feeding rate, water temperature and fish size. The empirical model was based on 129 measurements of average daily oxygen consumption rates of juvenile salmon. These measurements involved a variety of species and reflected a range of fish weights (0.65 - 33.3 g), temperatures T (5 - 20°C) and ration levels F (0 - 3.1%/d). Ration level was expressed as g of dry food per 100 g fish per day or %/day. Measured average daily Ro values ranged between 83 and 464 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$ and are plotted against the product $F*T$ in Figure 8C. A simple predictive equation for this set of data is given by: $Ro = 146 + 7.2*F*T$ ($r = 0.807$). In the discussion to follow, a more accurate model based on response surface analysis was used to predict Ro, and numerical results were generated using a computer program (Jensen et al. 1992). Predicted average daily values were multiplied by a factor to obtain the "safe Ro" value. This factor, which varied between 1.3 and 2, takes into account the diurnal variation in Ro and uncertainty in predicted average daily values. Safe Ro values were used in Equation 10 to calculate carrying capacity. This ensures that an oxygen criterion at the pond outflow is satisfied most of the time.

This model is used in a number of the illustrative examples to follow. It makes realistic predictions for juvenile fish in the range of experimental conditions previously indicated. Being an empirical model, it cannot be extrapolated beyond these conditions.

*c) Units for flow loading.* A number of methods have been developed to measure intensity in fish culture (Colt and Orwicz 1991*a*). In this discussion load rate Lr (biomass of fish per unit water flow) and density D (biomass per unit volume) are used. Units for Lr are kg per l/min. In terms of exponents this is kg $l^{-1}$ min. If 1000 kg of fish are supplied with 2000 l of water in 2 min, load rate is: 1000 kg/(2000 l / 2 min) = 1000 kg x 2 min/2000 l = 1 kg $l^{-1}$ min. Units for density are kg $m^{-3}$. Oxygen consumption Ro (and metabolite production) rates are expressed in terms of mg of oxygen per kg of fish per hour. If 2000 mg are consumed by 2 kg of fish in 4 h, Ro is 2000 mg/(2 kg * 4 h) = 250 $mg \cdot kg^{-1} \cdot h^{-1}$ or 250 mg/(kg h) — in terms of exponents this is $mg \cdot kg^{-1} \cdot h^{-1}$.

Equation 9 (Cf = Ci − Lr Ro/60) is dimensionally consistent with units of kg $l^{-1}$ min for Lr and $mg \cdot kg^{-1} \cdot h^{-1}$ for Ro. This can be shown by expressing units in exponent form and substituting into Equation 9. Noting that the factor 60 has units of min $h^{-1}$ and that Cf and Ci have units of mg $l^{-1}$, the equation simplifies to:

$$\text{mg } l^{-1} = \text{mg } l^{-1} - (\text{kg } l^{-1} \text{ min})(\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})/(\text{min h}^{-1}) \text{ or}$$

$$\text{mg } l^{-1} = \text{mg } l^{-1} - (\text{kg } l^{-1} \text{ min})(\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})(\text{min}^{-1} \text{ h}) \text{ so}$$

$$\text{mg } l^{-1} = \text{mg } l^{-1} - \text{mg } l^{-1}$$

Terms in the equation reduce to mg $l^{-1}$ and are therefore dimensionally consistent.

*2) Metabolite buildup.* Ammonia is a waste product of protein catabolism and is excreted via the gills (Wood 1958). Ammonia-N excretion rates ($R_N$) increase in response to feeding. Brett and Zala (1975) found that for sockeye salmon juveniles, rates increased from 8 $mg \cdot kg^{-1} \cdot h^{-1}$ before feeding to 35 $mg \cdot kg^{-1} \cdot h^{-1}$ after feeding (units are in mg ammonia-N per kg of fish per hour). Fish were fed between 0830 and 0930 h and ammonia excretion peaked about 4 hours later. McLean and Fraser (1974) measured

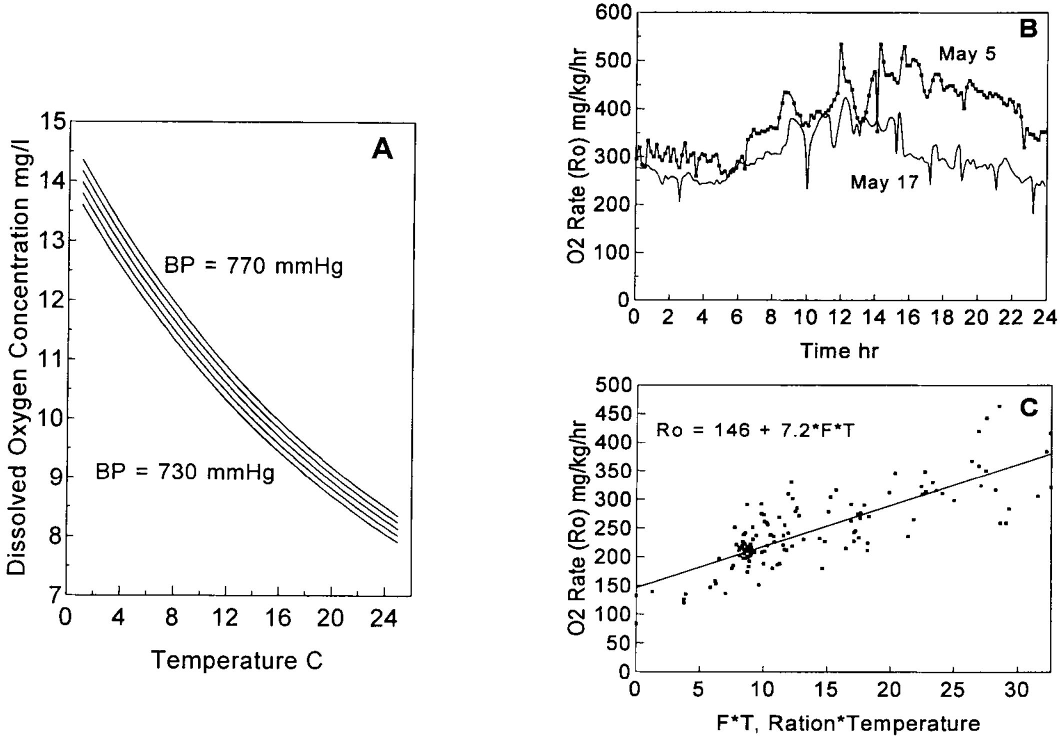

FIGURE 8. A. Dissolved oxygen concentration in air saturated water vs water temperature. Values are shown for barometric pressures varying from 730 to 770 mmHg. B. Variation in oxygen consumption rate (Ro) over a 24 hour period for a pond of chinook salmon juveniles at a low (May 17) and high ration level (May 5). Ro is in units of mg of oxygen per kg of fish per hour ($mg{\cdot}kg^{-1}{\cdot}hr^{-1}$). C. Average daily oxygen consumption rate ($mg{\cdot}kg^{-1}{\cdot}hr^{-}$) vs the product of ration level (%/day) and water temperature °C, F∗T.

ammonia-N production rates for coho salmon fingerlings in a production hatchery and found mean daily values varied between 8 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$ and 27 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$. With dawn to dusk feeding, ammonia excretion showed a daily pulse with the peak value occurring between 1500 and 1700 h.

Models relating excretion rate to feed rate have been reported by Willoughby et al. (1972), Speece (1973), Liao and Mayo (1974), BCR (1976), Hartman (1976), McLean (1979*a*), Fivelstad (1988) and Fivelstad et al. (1990). All models show a near linear increase with ration level. For 10-g fish at 10°C, average daily $R_N$ values ranged from 3 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$ at zero (0) ration to 22 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$ at maximum ration of 2%/d (McLean 1979*a*).

Aqueous ammonia exists in the un-ionized ($NH_3$) and ionized ($NH_4^+$) form. Un-ionized ammonia is the toxic component because it readily diffuses through biological membranes due to its lack of charge (Fromm and Gillette 1968). The proportion as un-ionized ammonia decrease as pH drops (Emerson et al. 1975). Figure 9A shows the percentage in the un-ionized form as a function of pH.

Small amounts of urea are also excreted as nitrogenous waste products (Wood 1958). Brett and Zala (1975) found that for sockeye salmon juveniles urea-N excretion rates were fairly constant. The daily average rate of 2.2 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$ accounted for 13% of the combined ammonia and urea excretion.

Although nitrite is not excreted by fish, it is an important water quality parameter in fish culture. Nitrifying bacteria oxidize ammonia to nitrite and so high concentrations can be found in rearing ponds. This is of particular concern where water is being re-used (Brown and McLeay 1975). As with ammonia, aqueous nitrite exists in the un-ionized ($HNO_2$) and ionized ($NO_2^-$) form. The un-ionized form is suspected of being the toxic component (Colt and Tchobanoglous 1976). Unlike ammonia, the proportion in the un-ionized form increases as the pH drops (Figure 9A).

Carbon dioxide is excreted at much higher rates than ammonia and also accumulates in rearing ponds. Using the concept of the Respiratory Quotient, R.Q.= ratio of carbon dioxide produced to oxygen consumed (Brett and Groves 1978), the carbon dioxide production rate $R_{CO_2}$ was related to Ro by: $R_{CO_2} = 1.238 * Ro$. The effect of increased carbon dioxide concentration on pH depends on the background alkalinity of the water supply and temperature (*see* page 421).

Suspended solids production rates, Rss (waste food and feces), are also related to feed rate and models have been proposed by Willoughby et al. (1972), Liao and Mayo (1974), Klontz et al. (1978), McLean (1979*a*) and Colt and Orwicz (1991*a*). Suspended solids generation is very difficult to predict because it is dependent on feeding efficiency and pond hydraulics. For 10-g fish at 10°C the predicted suspended solids production rate varied from 0 $\text{mg·kg}^{-1}\text{·h}^{-1}$ at 0 ration to 406 $\text{mg·kg}^{-1}\text{·h}^{-1}$ at a maximum ration of 2%/d (McLean 1979*a*).

The metabolite concentration at the outflow of a rearing pond containing B kg of fish and with water flow Q l/min (Lr=B/Q) was predicted by substituting the various production rates ($R_N$, $R_{CO_2}$, Rss) and initial concentrations into Equation 9. For example, if the ammonia-N concentration at the pond inflow is $C_{Ni}$ (mg/l) and the excretion rate is $R_N$ ($\text{mg·kg}^{-1}\text{·h}^{-1}$) then the outflow concentration $C_{No}$ is given by:

$$C_{No} = C_{Ni} + Lr\ R_N/60 \tag{11}$$

*3) Water quality criteria for rearing.* Setting water quality criteria for long term rearing is difficult because individual parameters interact in complex ways and interactions are poorly understood. Often criteria must be deduced from short-term toxicological studies involving a single parameter. In the rearing environment fish are exposed to a combinations of water quality parameters along with other stressors. This interplay occurs in the presence of pathogens and so precipitation of disease is always a threat. Because of these concerns, water quality limits for long term rearing must be set with a margin of safety. General criteria are viewed as tentative and must be tested by experience. Site specific factors such as background water quality, species, life stage and disease prevalence can invalidate general guidelines. Water quality guidelines for other life stages of salmonids are summarized in Chapters 2 and 5.

*a) Minimum DO.* As pond DO drops below saturation detrimental effects on the population increase. Setting minimum oxygen criteria involves a compromise between the efficient utilization of the water supply and fish health and growth.

Growth rate drops rapidly below a critical DO level. Coho juveniles at 20°C show a slow decrease in growth rate as DO falls below 8 mg/l followed by a rapid decrease as DO drops below the critical level of 5 mg/l (Brett 1979). Maximum swimming speed is also affected by DO. Coho juveniles are limited below 6 mg/l at 10°C and below saturation at 20°C (Fry 1971). Even when the water supply is fully saturated with oxygen the active metabolic rate is restricted at temperatures above 15°C (Brett 1972).

Minimum DO criteria must also take disease interactions into account. It is well known that even short episodes of low DO can precipitate furunculosis (Wedemeyer and McLeay 1981). Furthermore gill hyperplasia resulting from chronic exposure to metabolites can greatly increase the sensitivity of fish to low DO. Fish with gill hyperplasia can suffocate even when oxygen levels are near saturation.

Davis (1975) developed criteria that reflect levels of risk to a fish population. Level A satisfies the oxygen requirements of most of the individuals in a population, level B satisfies the average fish while level C meets the needs of only a small fraction of the population. At the lowest level most fish experience sublethal effects of oxygen deprivation.

These criteria are based on mass transfer of oxygen across the gills. Since the transfer rate depends on both the partial pressure gradient (between the ambient water and blood plasma) and the oxygen concentration of the ambient water, both parameters must be satisfied to meet a specific level of protection. Ambient oxygen concentration and partial pressure must be at least 7.75 mg/l and 120 mmHg to meet level A; 6 mg/l and 90 mmHg for level B and 4.25 mg/l and 60 mmHg for level C (Davis 1975). Values are shown for various temperatures in Table 4.

*b) Maximum metabolite concentrations.* Reported safe limits for un-ionized ammonia-N vary widely: 0.010 mg/l (Sigma 1983); 0.0125 mg/l (Colt and Orwicz 1991*a*); 0.04 mg/l (Meade 1985); 0.1 mg/l (Wickins 1980). The wide range in results may be due to the complex interplay of un-ionized ammonia ($NH_3$) with other water quality factors. Lowering ambient pH with $CO_2$, as opposed to acids such as HCl, does not produce the expected drop in ammonia toxicity (Lloyd and Herbert 1960; Warren and Schenker 1962).

Szumski et al. (1982) have modelled conditions at the surface of the gill and showed that the ambient $CO_2$ level effects un-ionized ammonia concentration. $CO_2$ excreted at the gill lowers the local pH and protects the gill by lowering the proportion of ammonia in the un-ionized form. However the magnitude of the pH drop is much greater when the ambient $CO_2$ levels are low. This occurs because the pH drops as the logarithm of the $CO_2$ concentration increases (*see* page 421 and Figure 9C). If ambient $CO_2$ is high, the proportional increase in $CO_2$ at the gill surface due to excretion is small and the decrease in pH is also small. Hence there is only a slight decrease in the un-ionized ammonia concentration at the gill. This is why un-ionized ammonia is more toxic when ambient $CO_2$ concentrations are high. Szumski et al. (1982) argues that many of the contradictions in ammonia results can be resolved by analyzing conditions at the surface of the gill rather than in the bulk solution. Erickson (1985) evaluated a number of ammonia toxicity models. The gill surface model was rejected after failing to explain a number of experimental results and a new model was proposed where both un-ionized and ionized ammonia are considered to be toxic. This joint toxicity model apparently explained many of the contractions in ammonia toxicity data.

This analysis implies that the present ammonia toxicity model is incomplete — toxicity cannot simply be predicted by calculating the concentration of ambient un-ionized ammonia from pH and temperature (Meade 1985). There is still major uncertainty with the present models of ammonia/$CO_2$ interaction (Colt and Orwicz

TABLE 4

Carrying capacities in kg of fish per l/min of water flow (MaxLr) at various fish weights, temperatures and ration levels. Values are generated to meet oxygen criteria (LvA, LvB and LvC)(Davis 1975) when the inflow saturation level is 95% and barometric is 760 mmHg. Average daily Ro values (mg·kg$^{-1}$·h$^{-1}$) are also shown. Details are given in McLean et al. 1993*a*.

| Wt g | Temp °C | Ration %dry/d | MaxLr LvA kg/lpm | DO LvA mg/l | MaxLr LvB kg/lpm | DO LvB mg/l | MaxLr LvC kg/lpm | DO LvC mg/l | Ro mg·kg$^{-1}$·h$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 0 | 1.05 | 9.7 | 2.10 | 7.3 | 3.15 | 4.8 | 79 |
| 1 | 5 | 1.07 | 0.51 | 9.7 | 1.02 | 7.3 | 1.53 | 4.8 | 198 |
| 1 | 5 | 2.14 | 0.40 | 9.7 | 0.80 | 7.3 | 1.19 | 4.8 | 262 |
| 10 | 5 | 0 | 1.35 | 9.7 | 2.69 | 7.3 | 4.04 | 4.8 | 54 |
| 10 | 5 | 0.50 | 0.74 | 9.7 | 1.48 | 7.3 | 2.22 | 4.8 | 126 |
| 10 | 5 | 0.99 | 0.57 | 9.7 | 1.15 | 7.3 | 1.73 | 4.8 | 171 |
| 20 | 5 | 0 | 1.52 | 9.7 | 3.04 | 7.3 | 4.57 | 4.8 | 44 |
| 20 | 5 | 0.39 | 0.84 | 9.7 | 1.68 | 7.3 | 2.52 | 4.8 | 107 |
| 20 | 5 | 0.79 | 0.64 | 9.7 | 1.27 | 7.3 | 1.91 | 4.8 | 152 |
| 1 | 10 | 0 | 0.79 | 8.6 | 1.60 | 6.4 | 2.41 | 4.3 | 96 |
| 1 | 10 | 2.13 | 0.30 | 8.6 | 0.62 | 6.4 | 0.93 | 4.3 | 301 |
| 1 | 10 | 4.27 | 0.24 | 8.6 | 0.49 | 6.4 | 0.74 | 4.3 | 382 |
| 10 | 10 | 0 | 1.02 | 8.6 | 2.07 | 6.4 | 3.11 | 4.3 | 67 |
| 10 | 10 | 0.99 | 0.44 | 8.6 | 0.89 | 6.4 | 1.34 | 4.3 | 200 |
| 10 | 10 | 1.98 | 0.32 | 8.6 | 0.65 | 6.4 | 0.98 | 4.3 | 283 |

TABLE 4 CONT'D.

| Wt g | Temp °C | Ration %dry/d | MaxLr LvA kg/lpm | DO LvA mg/l | MaxLr LvB kg/lpm | DO LvB mg/l | MaxLr LvC kg/lpm | DO LvC mg/l | Ro mg·kg$^{-1}$·h$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| 20 | 10 | 0 | 1.16 | 8.6 | 2.35 | 6.4 | 3.54 | 4.3 | 55 |
| 20 | 10 | 0.79 | 0.49 | 8.6 | 0.99 | 6.4 | 1.49 | 4.3 | 176 |
| 20 | 10 | 1.57 | 0.35 | 8.6 | 0.70 | 6.4 | 1.06 | 4.3 | 261 |
| 1 | 15 | 0 | 0.51 | 7.8 | 1.02 | 6 | 1.52 | 4.3 | 137 |
| 1 | 15 | 3.02 | 0.18 | 7.8 | 0.35 | 6 | 0.53 | 4.3 | 448 |
| 1 | 15 | 6.04 | 0.15 | 7.8 | 0.30 | 6 | 0.45 | 4.3 | 531 |
| 10 | 15 | 0 | 0.67 | 7.8 | 1.33 | 6 | 1.98 | 4.3 | 97 |
| 10 | 15 | 1.40 | 0.25 | 7.8 | 0.50 | 6 | 0.75 | 4.3 | 310 |
| 10 | 15 | 2.80 | 0.19 | 7.8 | 0.37 | 6 | 0.55 | 4.3 | 434 |
| 20 | 15 | 0 | 0.78 | 7.8 | 1.53 | 6 | 2.29 | 4.3 | 80 |
| 20 | 15 | 1.11 | 0.28 | 7.8 | 0.56 | 6 | 0.83 | 4.3 | 276 |
| 20 | 15 | 2.23 | 0.20 | 7.8 | 0.39 | 6 | 0.58 | 4.3 | 410 |

1991*a*). The resolution of this question has implications for fish culture because the low pH that occurs in rearing ponds is most often caused by elevated $CO_2$.

There are various sublethal effects associated with chronic exposure to low levels of ammonia in the fish culture environment. These effects are difficult to prove and most likely involve complex interactions with other metabolites such as suspended solids and carbon dioxide. Long-term exposure to ammonia is associated with gill hyperplasia and is thought to be a precursor to bacterial gill disease (Burrows 1964; Wedemeyer and McLeay 1981).

Dissolved oxygen concentration also modifies ammonia toxicity. Merkins and Downing (1957) showed that toxicity of $NH_3$-ammonia increased as oxygen decreased. In rearing ponds, high ammonia is usually found in conjunction with depressed oxygen. Ammonia toxicity may also be increased when the concentration of sodium ions in the water supply is low (Sigma 1983). In the discussion to follow, the maximum acceptable un-ionized ammonia-N concentration is 0.012 mg/l.

Long term exposure to high levels of carbon dioxide may cause nephrocalcinosis (calcium deposits in kidney) (Smart et al. 1979). Chronic high levels also leads to increased stress on the fish's oxygen transport system (Randall et al. 1982). Effects are much more severe if dissolved oxygen levels drop below 6.5 mg/l. Colt and Orwicz (1991*a*) have proposed at carbon dioxide criterion of 20 mg/l.

pH reductions can be severe in heavily loaded ponds if the background alkalinity of the water supply is low. Wickins (1980) proposes a minimum pH of 6.0 to 6.5. Colt and Orwicz (1991*a*) give a criterion of 6.0 to 9.0 but point out that the diel drop in pH may be more important than the minimum value. Sigma (1983) proposes an acceptable range for long term rearing of 6.5 to 8.5.

Suspended solids (waste food, feces) generated in rearing ponds are particularly irritating to gills. Unlike inorganic silt and clay, these particles have a high protein content and rapidly decompose to produce ammonia (Peel 1983). Chronic exposure to low levels can precipitate bacterial gill disease (Banks et al. 1979). This is a serious problem with newly ponded fry. Feeding is inefficient and their gills seem to be sensitive to particulates. Wickins (1980) proposes a safe level of 15 mg/l. This value has been adopted tentatively by Colt and Orwicz (1991*a*).

Nitrifying bacteria produce nitrite from ammonia and urea. Nitrite reduces total haemoglobin and converts haemoglobin to methaemoglobin thereby reducing the oxygen transport capability of blood (Brown and McLeay 1975). In this study the 96-hour $LC_{50}$ for rainbow trout was 0.23 mg/l $NO_2$-N. Safe levels for hard water are reported to be 0.10 mg/l (Wickins 1980). Sigma (1983) recommends a maximum limit of 0.015 mg/l. This discrepancy may result from the fact that calcium and especially chloride alleviate nitrite toxicity (Bath and Eddy 1980).

Water quality guidelines used in the discussion to follow are summarized in Table 5.

*c) Total gas pressure.* Excess gas pressure is normally measured in hatcheries using a portable saturometer. Excess pressure (or $\Delta P$) is the difference between the total gas pressure in the water and the barometric pressure (BP), $\Delta P = TGP - BP$ in mmHg. Traditionally total gas pressure is expressed as a percentage of the barometric pressure:

$$TGP\% = [(\Delta P + BP)/BP] * 100 \qquad (12)$$

If $\Delta P$ is greater than 0 or TGP% > 100% then the water is supersaturated. If oxygen and nitrogen percentage saturation levels are $O_2\%$ and $N_2\%$ then a useful approximation is $TGP\% = 0.21 * O_2\% + 0.79 * N_2\%$. The $\Delta P$ experienced by the fish decreases as water depth increases because hydrostatic pressure compensates for excess gas pressure in the water. A column of water 1 m deep exerts a pressure of 73 mmHg so if $\Delta P$ is 73 mmHg (TGP% = 110% at BP = 760) and fish hold at a depth of 1 m, the uncompensated value $\Delta Pu$ or excess gas pressure experienced by the fish is 0 mmHg, $\Delta Pu = \Delta P - 73 \cdot D$ (Colt 1984). Fish at the surface would experience a $\Delta P$ of 73 mmHg or a TGP% of 110%. It is the uncompensated gas pressure that causes gas bubble trauma and so fish constrained to shallow water are more sensitive to TGP. Response to gas supersaturation also depends on the gas composition or ratio of oxygen to nitrogen $O_2\%/N_2\%$. For a given TGP, toxicity increases as nitrogen increases and the oxygen to nitrogen ratio decreases.

These variables ($\Delta P$, $O_2\%/N_2\%$, depth) along with fish size and temperature have been used to predict biological response (Jensen et al. 1986). This model calculates $ET_{50}$ or estimated time (in days) to 50% mortality (Jensen et al. 1992). For fish culture purposes, this predictor can be interpreted as the relative risk imposed by a set of conditions. Low $ET_{50}$ values (< 10 d) indicate acutely lethal conditions and as $ET_{50}$ increases the risk of gas bubble trauma decreases (McLean et al. 1991).

$ET_{50}$ values are shown as a function of oxygen level in Figure 9B. Values beyond 100 days indicate negligible risk. Curves are plotted for nitrogen levels of 90, 95, 100, 103 and 106% of saturation to simulate conditions that could occur during oxygen supplementation. The model predicts that if nitrogen has been stripped to 90% then oxygen can be raised to very high values (200%) without the risk of gas bubble trauma ($ET_{50}$ > 100 d). However if background nitrogen is high (no stripping) the allowable oxygen increase is extremely limited. If nitrogen = 103%

**TABLE 5**

Water quality guidelines for rearing.

| Parameter | Limit mg/L |
|---|---|
| Ammonia-N | 0.012 mg/L |
| Nitrite-N | 0.015 mg/L |
| pH | 6.5 to 8.5 |
| Carbon Dioxide | 20 mg/L |
| Suspended Solids | 15 mg/L |
| Dissolved Oxygen | *see* Table 4 |

and oxygen = 160% $ET_{50}$ is only 20 d (Figure 11B). These conditions pose a significant risk and would not be suitable for long term rearing.

There may be additional problems for fish suddenly released from high oxygen ponds to the natural environment with perhaps half the oxygen concentration. Exposure to high oxygen levels for long periods is known to reduce hematocrit levels. There may be an adjustment period as the oxygen carrying capacity of the blood slowly increases. During this period fish may be more vulnerable to predation.

General TGP criteria that attempt to establish safe values over a wide range of conditions have been proposed for fish culture. Sigma (1983) limits TGP% to a maximum of 103%. Colt et al. (1991) give a preliminary safe limit for $\Delta P$ of 10 mmHg. This level is designed to protect early juveniles — the most sensitive life stage. The maximum level for oxygen is 300 mmHg (191% saturation or 21 mg/l at 10°C). Fidler and Miller (1993) base safe limits on the threshold for swim bladder over-inflation of small fish in shallow water. This is the first response to elevated gas pressures and occurs when $\Delta P > 73.4 \cdot D + 0.15 \cdot pO_2$, where $pO_2$ is the partial pressure of oxygen (157 mmHg at 100% saturation). A general hatchery criterion is derived by setting $D = 0$ and $pO_2 = 157$ mmHg — the $\Delta P$ limit is $0.15 \cdot 157$ mmHg = 24 mmHg (TGP% = 103%). It should be noted that a TGP of 103% is highly conservative when the oxygen to nitrogen ratio is high.

*4) Model synthesis.* The maximum loading rate (carrying capacity of the water supply) with respect to each metabolite can be calculated from Equation 10. Metabolite production rates (Ro, $R_N$, $R_{CO_2}$, Rss), inflow (background) concentrations, the minimum acceptable oxygen concentration and maximum acceptable metabolite concentrations are substituted into Equation 10 so that the maximum Lr value for each parameter is

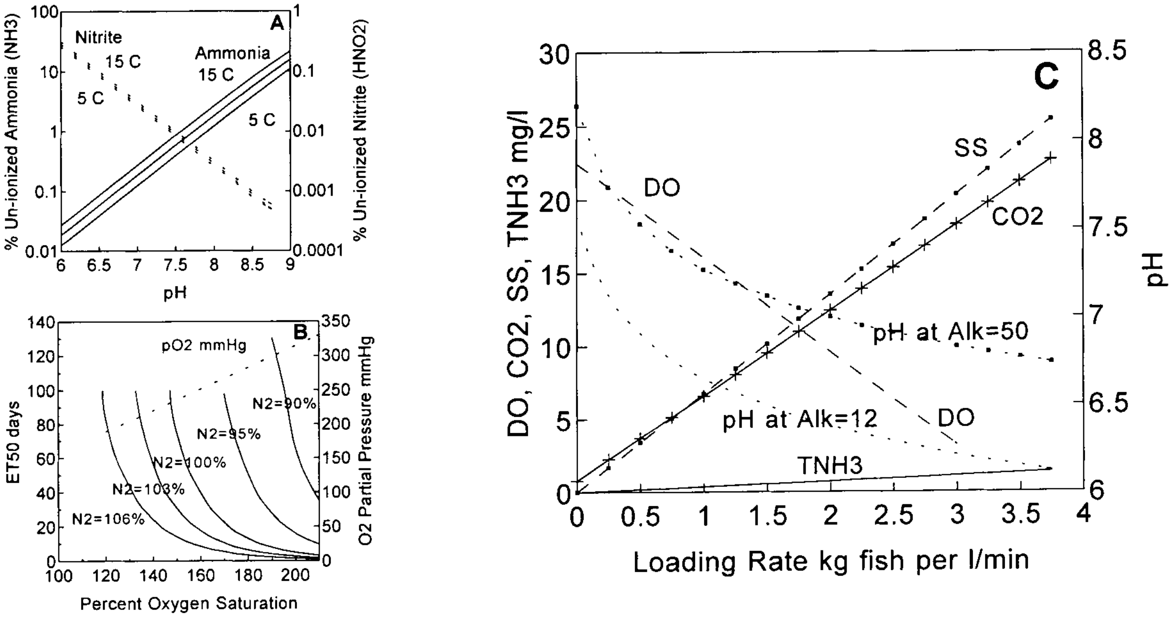

FIGURE 9. A. Percent un-ionized ammonia and nitrite vs pH at water temperatures of 5, 10 and 15°C. B. Estimated time to 50% mortality ($ET_{50}$) vs percent oxygen saturation. Curves are plotted for various levels of nitrogen saturation %. Partial pressure of oxygen (mmHg) is also shown. C. Dissolved oxygen, carbon dioxide, suspended solids (SS) and total ammonia-N concentration (mg/l) at the outflow of a rearing pond vs loading rate (kg of fish per l/min). Inflow conditions are as follows: DO = 200% of saturation (22.5 mg/l), $CO_2$ = 0.8 mg/l (air saturated), SS and ammonia-N = 0 mg/l. Outflow pH is shown for background alkalinities of 12 and 50 mg/l as $CaCO_3$.

predicted. The parameter which yields the lowest Lr value is the first limiting factor and determines the carrying capacity of the water supply.

Dissolved oxygen is commonly the first limiting factor in fish culture. Carrying capacities based on this assumption are shown in Table 4 over a range of culture conditions. These calculated values assume a barometric pressure of 760 mmHg, zero salinity and an inflow oxygen saturation of 95%. Average daily oxygen consumption rates (Ro) are also shown. Diurnal variation in Ro and statistical uncertainty in predicted values are taken into account so these loads are considered "safe" — i.e., specified oxygen criterion will be satisfied most of the time (*see* page 407).

*Other Limitations.*

*1) Water velocity.* The water flow rate, along with the shape of the pond and nature of the inflow and outflow structures, determines the distribution of flow and the magnitude of water velocities experienced by the fish. The average water velocity in a raceway is given by $U = 16.67*(Q/A)$ where U = velocity in cm/s, Q = flow in l/min and A = cross sectional area $cm^2$. In a circulating type pond inflow water is introduced under pressure and a circulating current is set up in the pond. A wide range of velocities can be obtained depending on pond design and the orientation of the inflow jets (*see* page 397).

The sustained swimming speed imposed by the rearing container has important implications to fish health. Christiansen (1991) reported a complex array of behavioural and physiological effects of sustained swimming speed on salmonids. Compared to standing water conditions, fish reared at moderate swimming speeds of 1 to 2 FL/s (fork lengths/sec) showed: reduced aggressive behaviour (nipping), increased feeding activity, improved growth and increased disease resistance. Exercise induced by water velocity increased smolt stamina and survival rates following release (Burrows 1969; Burrows and Chenoweth 1970).

These benefits can easily be lost if velocity is too high. Above 2 FL/s growth may be slowed due to energy costs of swimming. For juvenile fish at 10°C the maximum 60 minute sustained (aerobic) swimming speed is approximately 4 to 5 FL/s (Brett and Glass 1973). Higher swimming speed requires anaerobic metabolism and can only be sustained for a short time period. Burst speeds result in oxygen debt and a build up of lactic acid in the muscle.

Optimum water velocity criteria for long term rearing have not yet been determined (*see* page 400). It is still not clear whether there are benefits of moderate velocity when sustained over the entire freshwater rearing phase. Periods of higher velocity at critical developmental stages (e.g., parr to smolt transformation) may be more beneficial (Christiansen 1991). Optimum swimming speed is also a function of temperature, species and fish size. This relationship has not been determined and so water velocity criteria for rearing are uncertain.

Pond velocities also have implications for how waste solids (uneaten food and feces) behave. Westers and Pratt (1977) recommended a maximum water velocity for raceways of 3.3 cm/s so that solids settle on the pond bottom. Suspended solids can cause gill irritation so rapid settling is advantageous. Once solids have settled they can be removed routinely using a vacuum (Peel 1983). This is labour intensive and can re-suspend solids. Baffles can also be used to concentrate the solids for ease of removal. They create high velocity areas along the pond bottom which move the solids to the pond outflow where they can easily be removed without the risk of re-suspension (Boersen and Westers 1986). Fixed baffles on small troughs have also been found to improve mixing and flow distribution (Wolfe 1983). A continuously moving baffle has been developed to make troughs essentially self cleaning (Abraham et al. 1985). They also create localized areas of high velocity thereby allowing fish to select a range of water velocities (Boersen and Westers 1986).

Circulating ponds on the other hand operate at high velocities so that solids remain suspended and are continuously flushed out of the pond. For example the 75-ft long rectangular circulating pond (Burrows and Chenoweth 1970) becomes self cleaning at flows above 1500 l/min. At this flow the velocity along the outer wall of the pond is 21 cm/s and solids are kept in suspension. At a maximum flow of 2271 l/min the velocity along the centre and outer walls are 9.4 and 33.8 cm/s respectively. This means that 5-g (7.9 cm) fish are forced to swim at between 1.2 and 4.3 FL/s to hold position in the current. Care must be taken with small fish in circulating ponds. Velocities required to make the pond self cleaning are near their maximum sustained swimming speed and are not suitable for long term rearing.

2) *Density* (*see also* page 388). Water flow requirements are effected by the biomass density (kg/m$^3$) in the pond. Increasing density causes many complex behavioural effects but it is generally agreed that high density is detrimental for most species. Stress, lowered disease resistance and lowered survival are associated with high density (Fagerlund et al. 1987). Abundant flow of good quality water (low loading rates) may reduce some of these effects (Colt and Orwicz 1991*b*). In experiments designed to test the extreme limits of high density, Buss et al. (1970) reared rainbow trout in vertical silos. It was reported that very high densities could be tolerated (137 kg/m$^3$) if water quality and flow rate were maintained at high levels. Alderdice (1980) also emphasized that excellent water quality is required to rear fish at high densities without disease.

Banks (1994) measured the effects of inflow rate and rearing density on subsequent survival rates of chinook smolts released to the Columbia River. Ponds with three times the density (13.7 vs 39.1 kg/m$^3$) but with the same water flow had a 76% lower survival rate. Because flow was constant the high density ponds also had a higher load rate (0.6 vs 1.71 kg per l/min). Although three times as many smolts were released from the high density pond the adult contribution was only 67% of the low density pond. In high density ponds where the flow was also increased three times (to maintain Lr = 0.6 kg per l/min), survival rate dropped by only 56% (compared to 76% lower survival rate when flow was the same). With additional flow the effects of density were somewhat reduced and the high density pond produced 1.38 times as many adults as the low density pond.

*3) Pathogen load.* The presence of disease organisms can increase the water flow requirements for rearing. Reduced water quality increases levels of stress and lowers disease resistance (Wedemeyer and McLeay 1981). If the disease organism is present at sufficient density, environmental degradation usually leads to an epizootic. This relationship is intensified at higher temperatures and if other stressors such as handling are present.

*4) Background water quality.* Along with dissolved oxygen, metabolite and total gas pressure requirements, there are a number of other water quality parameters that have important effects on rearing. Temperature is a master variable that affects every aspect of rearing. The upper lethal limit for Pacific salmon is between 24 and 26°C (Brett 1952). However optimum growth and survival occurs at much lower temperatures. The upper limit for successful smoltification and downstream migration of coho and chinook salmon is 15°C (Wedemeyer et al. 1981). Maximum growth for sockeye juveniles fed to satiation occurs at 15°C (Brett et al. 1969) while the maximum for chinook fingerlings (satiation ration) occurs at 19°C (Brett et al. 1982). It is interesting to note that if ration was reduced to 60% of satiation the optimum temperature for growth dropped from 19 to 15°C. At this reduced ration there was no growth at 21.4°C — all available energy was going into maintenance (*see also* Chapter 7).

There are a number of beneficial ions that mitigate the effects of metabolites and rearing stress (*see* pages 411 and 414). Although there are no well established guidelines, extremely low concentrations of these may increase the carrying capacity of a water supply. In some cases salts must be added continuously in order to increase ionic strength.

The effects of ammonia are reduced if the sodium concentration is equal to or greater than the total ammonia concentration (Sigma 1983). Chloride reduces nitrite toxicity (Wedemeyer and Yasutake 1978). Protection is afforded if chloride is 100 times the nitrite-N concentration (in units of mg/l) (Sigma 1983). These ions added at 1000 to 5000 mg/l also help alleviate handling and transport stress (Wedemeyer 1972; *see* Chapter 12).

Hardness is a measure of the concentration of divalent cations and is expressed in units of mg/l as calcium carbonate. In natural waters most hardness is contributed by calcium and magnesium. The relationship between hardness in mg/l as $CaCO_3$ and calcium and magnesium in mg/l is:

$$\text{hardness} = 2.5 * \text{Ca}^{2+} + 4.11 * \text{Mg}^{2+} \qquad (13)$$

Calcium reduces the impact of: low pH (acidic conditions) (Randall et al. 1982), high nitrite (Wedemeyer and Yasutake 1978), and metals such as copper and zinc (Sigma 1983). It has also been found to reduce severity of a number of fish diseases. Warren (1963) found a statistical correlation between water hardness and bacterial kidney disease. Hatcheries with hard water (> 300 mg/l) had no kidney disease while soft water facilities (< 10 mg/l) had a good chance of having a yearly outbreak. Jensen (1980) found that calcium reduced the severity of gas bubble disease in steelhead fry while McLean (1979*b*) showed that calcium addition (hardness levels increased from 18 to 49 and 90 mg/l) reduced the incidence of coagulated yolk in steelhead alevins.

Alkalinity is the acid neutralizing capacity of the water and is usually correlated with hardness in natural waters. It also has units of mg/l as calcium carbonate. $CO_2$ excretion can lower the pH of waters with low alkalinity. The magnitude of the pH drop can be approximated using the first ionization constant of aqueous carbon dioxide $K_1$. Final pH can be calculated from $K_1$ (Sawyer and McCarty 1967; Sigma 1983) or read from nomographs (APHA 1980). This method assumes that the carbonate/bicarbonate system is the main regulator of pH and that pH is below 8.5 (Stumm and Morgan 1970). Background alkalinity is expressed in traditional units of mg/l as calcium carbonate and the equilibrium constant for aqueous $CO_2$ must be corrected for temperature and ionic strength.

At 10°C in low ionic strength water,

$$K_1 = [H^+][HCO_3^-] / [H_2CO_3] = 3.436*10^{-7} \qquad (14)$$

(Stumm and Morgan 1970). In this expression square brackets denote concentration units of moles/l and $H_2CO_3$ refers to total analytical concentration of dissolved carbon dioxide. If the bicarbonate alkalinity is 100 mg/l as $CaCO_3$ (0.002 moles/l of bicarbonate) and the $CO_2$ concentration is 10 mg/l ($2.27*10^{-4}$ moles of $H_2CO_3$) then pH can be found by substituting the molar values into the expression for $K_1$ and solving for $[H^+]$,

$$pH = -\log[H^+] = -\log(3.90*10^{-8}) = 7.4 \qquad (15)$$

A useful expression for pH is:

$$pH = pK_1 - \log([H_2CO_3]/[HCO_3^-]) \qquad (16)$$

where $pK_1 = -\log K_1 = 6.464$. If the water supply has an alkalinity of only 15 mg/l as $CaCO_3$ (typical surface water of coastal B.C.), 10 mg/l of $CO_2$ will decrease the pH to 6. If the alkalinity is too low, excretion of $CO_2$ depresses pH and limits carrying capacity.

It must be emphasized that this model does not take into account other acids or bases that may be present. For example excreted ammonia is a weak base and would therefore counter the pH drop caused by $CO_2$. At levels normally found in fish culture the effect is small but if the ammonia concentration is high, pH values predicted by this simplified model will be too low. It should also be noted that at pH values above 8.5 the second ionization constant of aqueous carbon dioxide must also be taken into account.

*Optimum Carrying Capacity.*

*1) Fish farming.* Water flow must be sufficient to maintain environmental criteria as discussed previously. However maximum loads based on these considerations must be tested in the production setting and may require modification if higher growth rates are required or disease outbreaks become unmanageable.

Table 4 gives carrying capacity (kg of fish per l/min) for good quality water at different temperatures and fish sizes if the inflow oxygen concentration is 95% of saturation and the ration is maximum. It is recommended that oxygen concentration at the outflow be kept above 6 mg/l and 90 mmHg (Level B in Table 4).

These values are not realistic if the water quality is initially poor (e.g., high background ammonia, carbon dioxide or suspended solids). They may also be inaccurate if feed rates exceed the appetite of the fish or when feeding is initiated in newly emerged fry. It is also assumed that the flow is uniformly distributed so that the outflow has the lowest oxygen concentration in the pond. If flow distribution is poor, the predicted water flow requirements will not protect the fish -- localized areas in the pond may have lower oxygen concentrations than predicted by Table 4.

*2) Ocean ranching.* Cumulative effects of high density and degraded water quality during juvenile rearing are a special concern in ocean ranching. Quality is of prime importance because success is measured by the ability of smolts to make the transition to seawater and ultimately by the contribution of adults over succeeding years. If N smolts are released from a pond and their survival rate is S, then the goal is to maximize the adult contribution, or $N*S$. However as N increases the effects of crowding become more severe and the chance of survival may decrease. The functional relationship between S and N is extremely complex and may be impossible to quantify with much precision.

After smolts are released they become part of a larger ecosystem where they must compete for food and avoid predators. Smolt survival is affected by so many variables that the role of physiological quality (and hence rearing density) may be masked. For example Bilton et al. (1982) found that coho salmon survival was extremely sensitive to both release time and the average weight of smolts at release. In this experiment all groups of smolts were reared for approximately one year and were physiologically capable of making the transition to seawater. At Rosewall Creek the survival rate of smolts released in mid-June was over five times greater than releases in mid-April. Although the mechanism was not investigated, this empirical study showed that good quality smolts released at the wrong time or size failed to achieve their full potential for survival.

Since this release window is related to estuarine and marine conditions it is likely that the optimum varies between sites and from year to year. Peterman (1980) argues that a number of density-dependent factors may be operating so that survival rate is influenced by the total population of migrating smolts and not simply by pond population. This could occur if estuarine capacity or marine food supplies were limiting or if predators aggregated in response to an increased abundance. Peterman (1980) also points out that ocean conditions are dynamic and so survival relationships can be expected to vary with time. Not only is the web of variables affecting smolt survival highly complex and dynamic, measurement of survival rate itself is very problematic. Cross et al. (1991) describes the methodology employed to measure total survival rate, and highlights uncertainties and weaknesses in the data.

In face of these difficulties, the most practical approach is to set water flow rates so that the most stringent water quality criteria are satisfied and high quality smolts are produced. It is recommended that oxygen concentration at the outflow be kept above 7.8 mg/l and 120 mmHg (Level A in Table 4).

This conservative approach is justified by experimental results. Banks (1994) performed a 3X3 multifactor experiment involving fish density and water flow rate. Raceways (volume = 34.2 $m^3$) were loaded with 20,000, 40,000 and 60,000 chinook salmon and operated at water flows of 757, 1514 and 2271 l/min to give nine experimental treatments. The temperature over the 9-month rearing period was fairly stable at 6.4°C. The average weight of smolts at release was 25 g so densities reached approximately 14, 27 and 39 kg of biomass per $m^3$ and flow loading rates varied from 0.22 to 1.92 kg per l/min. Smolt survival rates dropped as water flow dropped and Lr increased. At all densities tested, the highest survival and contribution of adults occurred with the highest water flow rate. A satisfactory mechanism could not be found to explain these results. Water quality was reasonably good in all treatments and fish behaviour and growth were also normal. It was proposed that the higher flows (lower Lr) may have resulted in subtle advantages in disease control and pond hydraulics (cleanliness). There is some evidence that these advantages may have produced smolts that were more adaptable to seawater (*see also* page 419).

### *Summary of Useful Rearing Pond Relationships.*

There are a number of relationships between pond dimensions, flow and fish loading that are useful when discussing rearing intensity. Consider a pond with cross-sectional area A, length L and flow Q containing a biomass of B kg. For simplicity, relationships are derived without reference to units. Pond volume V, average water velocity U, loading rate Lr and density D are given by: $V = A*L$; $U = Q/A$; $Lr = B/Q$; $D = B/V$. The theoretical mean residence time for water in the pond T and exchange rate Er (complete exchanges per unit time) are given by $T = V/Q$ and $Er = 1/T = Q/V$. Substituting $Q = U*A$ and $V = A*L$ into the expression for exchange rate gives Er =

$U*A/(A*L) = U/L$. Solving for mean velocity in terms of Er and pond length L gives $U = Er*L$. Dividing density by load rate gives $D/Lr = (B/V)/(B/Q) = Q/V = Er$. Therefore density is related to exchange rate and load rate: $D = Er*Lr$.

The expression for oxygen (or metabolite) concentration at the pond outflow Cf can be expressed in a number of equivalent ways: in terms of load rate Lr, $Cf = Ci - Ro\ Lr/60$ (*see* Equation 9); in terms of mean residence time T (h), $Cf = Ci - Ro\ D\ T/1000$; and in terms of exchange rate Er (per hour), $Cf = Ci - Ro\ D/(1000\ Er)$.

Westers and Pratt (1977) used these relationships to generate guidelines for the design of raceways. Firstly the relative dimensions, length to width to depth, are set at 30:3:1. Secondly the maximum load rate Lr (kg of fish per l/min) is determined in order to satisfy the metabolic demands of the fish. At this point total biomass and pond flow have not been determined. Thirdly the exchange rate Er is set at four per hour. With Er set, the maximum density can be derived from $D = Er*Lr$. With units of kg per l/min for Lr, the maximum fish density is: $D = 16.67*Er*Lr$ and with $Er = 4$, $D = 55.7*Lr$. Lastly the average velocity U is limited to 0.033 m/sec so that particulates can settle (minimizing gill damage). Mean velocity is related to optimum pond length by $U = Er*L$ and with units of m/sec is given by $U = Er*L/3600$. With $Er = 4$ and $U = 0.033$ m/s, the optimum length is $L = 118.8/Er = 30$ m. Using the relative pond dimensions, total volume V is 90 $m^3$ and the cross sectional area A is 3 $m^2$. Total flow is found from $Q = U*A = 0.033*3 = 0.099\ m^3/s = 5940$ l/min. The maximum biomass is given by $B = D*V = (55.7*Lr)*90 = 5013*Lr$. With constraints on relative pond dimensions, exchange rate and average velocity, the operating characteristics and dimensions of a raceway can be calculated.

The relationships given above are theoretical and must be applied with caution. Rosenthal (1986) points out that the interpretation of mean residence time depends on pond type (mixed vs raceway) and that large deviations often occur between theoretical and actual exchange rates (*see* page 392). In an ideal raceway the entire pond volume is replaced after one mean residence time T has elapsed. For example, if raceway flow is shut off and a treatment chemical is mixed into the pond at concentration Co, the chemical is entirely flushed out T minutes after the flow has been turned back on, i.e., concentration $C = 0$ at $t = T$. In an ideal mixed flow pond the concentration C drops off exponentially as the clean water dilutes the chemical:

$$C = Co \cdot e^{(-t/T)} \qquad (17)$$

After one mean residence time ($t = T$) the concentration drops to $0.368*Co$ or 36.8% of the initial chemical concentration. In other words 63.2% of the original tank volume has been replaced with clean water. Deviations from these ideal models arise because of poor mixing, short circuiting of flow caused by packing or movement of the fish and by jet effects of the inlet structure. These problems can be more critical as the size of the rearing pond increases.

## OXYGEN SUPPLEMENTATION

The carrying capacity of the water supply can be increased if oxygen supplementation or re-use technology are used. The simplest way to augment oxygen supply is to aerate the incoming water using a conventional aeration tower or packed column. If these devices are operated at atmospheric pressure it is usually possible to increase the oxygen concentration to 95% of saturation. To achieve further increases more sophisticated oxygen supplementation technology must be used. These systems use pressurized air or pure oxygen and can achieve dissolved oxygen levels up to 200% of saturation. As the loading rate increases other metabolites become limiting and benefits of further oxygen supplementation cease. At this point increases in carrying capacity can only be achieved by treating and re-using the water. Depending on a number of site-specific factors, re-use may require suspended solids, nitrogen gas, ammonia or carbon dioxide removal as well as aeration.

### *Uses of Oxygen Supplementation*

*Increased Carrying Capacity.* Oxygen supplementation can be used to increase the carrying capacity if oxygen is the first limiting factor. This is its main purpose in fish farming where biomass harvested is the prime objective. It has proven to be a more economical method of increasing production than developing new water supplies and pumping more water.

Colt and Orwicz (1991*b*) have shown that the potential for increased production is determined by a complex interaction of site specific factors. These factors include the background water quality, the type of oxygen addition technology used, and the overall configuration of the fish production system. As noted previously (*see* Table 5), carbon dioxide, ammonia and suspended solids accumulations can also limit production and therefore must be considered.

These complex interactions will be explored later (*see* page 433) using a computer model. This model shows that increasing carrying capacity by addition of oxygen always results in a trade-off in environmental quality. This trade-off may not be acceptable where smolt quality is of prime importance — e.g., in ocean ranching where adult returns (rather than biomass produced in the pond) is the criteria for success.

*Removal of Nitrogen Gas.* Systems using pure oxygen strip nitrogen more effectively than simple aeration towers operating under atmospheric conditions. With these systems it is possible to reduce both nitrogen and total gas pressure to below 100% of saturation. This feature was an important reason for installing oxygenation systems in Michigan hatcheries (Westers et al. 1987).

Low persistent levels of gas supersaturation have been suspected of causing chronic stress in hatcheries for some time. Fish may be more susceptible to supersaturation in hatcheries because they are constrained to shallow water. In this case there is no mitigating effect of water depth and the fish experience the full excess gas pressure (*see* page 415).

Hatcheries using conventional aeration systems often have low chronic levels of gas supersaturation. Mean levels of 103% ($\Delta P = 23$ mmHg) are common. Fidler (1983) has pointed out that $\Delta P$ is not stable — in fact it fluctuates in response to changes in temperature and barometric pressure. If $\Delta P$ is 23 mmHg and the barometric pressure suddenly drops from 760 to 730 mmHg, the $\Delta P$ experienced by the fish increases to 53 mmHg (TGP% = 107%). The water supply eventually adjusts to the new barometric pressure but there is a time delay during which the fish experience an elevated $\Delta P$ of 53 mmHg. Oxygenation systems can reduce nitrogen below 100% and eliminate concerns over chronic exposure to supersaturation.

*Optimize Rearing Environment.* Oxygen can be introduced down the length of a raceway to eliminate the dissolved oxygen gradient that normally exists between inflow and outflow. This may improve fish distribution in the pond and help to improve the overall quality of the environment.

*Back-up or Emergency Life Support.* Oxygenation systems can be used in an emergency to compensate for a reduction in water flows. An oxygenation system was used to oxygenate a remote spawning channel during an emergency that developed in September 1989 (Seppala 1991). A large run of pink salmon returned to the Glendale system during drought conditions. Low flows coupled with high temperatures and large biomass caused the dissolved oxygen to drop to 2 mg/l. This lead to mass suffocation of adult fish. Ceramic stones and liquid oxygen tanks were transported to the site and provided life support for 70,000 salmon in a spawning channel during the most critical period. These systems can also act as a back-up for pumped water. If pumps fail, dissolved oxygen drops to critical levels within minutes. An oxygenation system can extend the survival time to many hours giving time for remedial action (McLean et al. 1993*a*).

### ***Methods of Adding Oxygen***

There is a wide range of aeration devices using both air and pure oxygen available to fish culturists (Colt and Orwicz 1991*a*). The simplest devices (screen decks, packed columns, surface paddles, aspirators, air stones) mix air and water to drive the individual gas components in the water supply (e.g.. oxygen, nitrogen, carbon dioxide, hydrogen sulfide) towards equilibrium. The most promising of these techniques have been intensively evaluated (McLean and Boreham 1980; Owsley 1981; Hackney and Colt 1982; Shrimpton 1987; Bouck et al. 1984; McLeod 1986; MacKinlay 1991).

As gas measurement techniques have improved and awareness of trauma caused by chronic supersaturation has increased (Wright and McLean 1985; Colt et al. 1986; Jensen et al. 1986; Fidler 1988; Shrimpton et al. 1990), the critical assessment of aeration systems has intensified. A number of the earlier methods (e.g., aspirators, submerged aerators) were found to create harmful levels of gas supersaturation (Colt and Westers 1982). The need for higher oxygen levels without concurrent gas supersaturation has lead to a host of new techniques involving pure oxygen.

*Basic Principles.* The rate at which oxygen dissolves in water exposed to the atmosphere is proportional to the difference between the saturation Cs and ambient concentration C. The difference, Cs - C, is the oxygen deficit D or driving force for the reaction. The rate of change in the deficit dD/dt is proportional to D and is described by the equation:

$$dD/dt = -kD \tag{18}$$

This is an empirical equation which is also valid for nitrogen. The proportionality constant k is determined by many factors including the type of aeration device, gas species and water temperature. Solution of this differential equation gives the final deficit Df at time t in terms of the initial deficit Di and the constant k:

$$Df = Di\ e^{-kt} \tag{19}$$

where $e = 2.718...$

This relationship can be expressed in terms of concentration, percent saturation or partial pressure. At a barometric pressure (BP) of 760 mmHg and a temperature of 10°C, freshwater in equilibrium with air has an oxygen concentration of 11.26 mg/l (100% saturation). At equilibrium the partial pressures of individual gases in the gas and liquid phases are in balance. Since oxygen occupies 20.946% of dry air, the partial pressure is $0.20946*(760 - 9.2) = 157.3$ mmHg (9.2 mmHg is water vapour pressure at saturation). This is the partial pressure of oxygen in air saturated water at 10°C (11.26 mg/l).

Nitrogen, the other major gas component, occupies 79.018% of dry air and so at its equilibrium concentration of 18.82 mg/l (100% saturation) exerts a partial pressure of $0.79018*(760 - 9.2) = 593.3$ mmHg. Carbon dioxide makes up only 0.033% of dry air and at equilibrium (0.78 mg/l) exerts a very small partial pressure of $0.00033*(760 - 9.2) = 0.25$ mmHg (CRC).

*Aeration Towers at Atmospheric Pressure.* Water is pumped to the top of an open tower and allowed to fall through screens or media (e.g., bio-rings). The flow must be distributed evenly and the column must be ventilated for good results. Performance is a function of the flow loading or flow per unit cross-sectional area of the column. If flow is too low the distribution system may not work and flow is short circuited. At high flows, the column floods and no gas transfer occurs. Since the column operates at atmospheric pressure all gases approach equilibrium. These devices are often used on groundwater to increase oxygen and remove nitrogen, carbon dioxide and hydrogen sulfide.

Groundwater is typically undersaturated in oxygen and supersaturated in nitrogen. If the ambient oxygen concentration is 2 mg/l (BP = 760 mmHg, temperature = 10°C) then % saturation is 2/11.26*100% or 17.76% and partial pressure is 157.3*0.1776 or 27.9 mmHg. This initial deficit Di can be expressed as: 11.26 − 2 = 9.26 mg/l; 100% − 17.76% = 82.24%; or 157.3 − 27.9 = 129.4 mmHg.

If this water supply is passed through a screen deck-type aeration column that is open to the atmosphere, oxygen approaches equilibrium and the deficit shrinks. If the column consists of n perforated aluminum screens spaced 10 cm apart, and the aeration constant k for oxygen is 0.11 per screen (McLean and Boreham 1980), the final deficit Df is given by Di $e^{(-0.11*n)}$. Column performance for n = 10 and 20 screens is summarized in Table 6.

With initial oxygen Ci at 2 mg/l (17.76%) and the number of screens doubled from 10 to 20, the final oxygen concentration Cf increases from 8.18 mg/l (72.63%) to 10.23 mg/l (90.89%). The driving force, initially very large Di = 129.4 mmHg, diminishes as the oxygen concentration increases and so adding more oxygen becomes prohibitively expensive. Even if the pumping head is doubled and the tower height is increased to 40 screens, the oxygen concentration is only increased by 0.92 mg/l (10.23 to 11.15 mg/l).

*Pressurized Systems.* Oxygen addition can be enhanced if the partial pressure in the gas phase is increased. This occurs when air bubbles are carried to a water depth or when an aeration tower is sealed and oxygen gas or air are introduced under pressure. If an aeration column is sealed and the air pressure is increased to 1.1 Atm or 836 mmHg the oxygen partial pressure in the gas phase increases from 157 to 173 mmHg and the nitrogen increases from 593 to 653 mmHg. If water is 60% saturated in oxygen (94 mmHg) and 120% in nitrogen (712 mmHg) (typical ground water), the driving force for oxygen addition increases from 63 mmHg (157 − 94) in the open column to 79 mmHg (173 − 94) in the pressurized system. Unfortunately the driving force for nitrogen stripping decreases from 119 mmHg (712 − 593) to 59 mmHg (712 − 653) in the pressurized column.

This problem can be eliminated if pure oxygen instead of air is used in the column. In this case the partial pressure of oxygen is equal to the total pressure (836 mmHg) and the nitrogen partial pressure in the gas phase is 0 mmHg. The oxygen

TABLE 6

Oxygen concentration mg/l, % saturation and partial pressures before (Ci) and after (Cf) aeration. Deficits before (Di) and after (Df) treatment are also shown. Water was passed through columns having 10 and 20 screens.

| | Before treatment | | After treatment | | | |
|---|---|---|---|---|---|---|
| | | | 10 screens | | 20 screens | |
| | Di | Ci | Df | Cf | Df | Cf |
| Concentration mg/l | 9.26 | 2.00 | 3.08 | 8.18 | 1.03 | 10.23 |
| Saturation % | 82.2 | 17.8 | 27.4 | 72.6 | 9.1 | 90.9 |
| Partial P mmHg | 129.4 | 27.9 | 43.1 | 114.2 | 14.3 | 143.0 |

gradient is greatly increased (836 − 94 = 742 mmHg) and so the transfer rate to the water is increased. Nitrogen stripping is also enhanced — the gradient between the gas phase and water is 712 − 0 = 712 mmHg and so nitrogen is readily removed from the water supply.

1) *Fine bubble diffusers*. Direct injection of fine bubbles is one of the simplest and most flexible oxygenation methods. Fine bubble diffusers are used to force small bubbles of oxygen directly into a water supply. High quality ceramic diffusers give uniformly small bubble diameters (4 $\mu$m) so that surface area and hence gas transfer rates are maximized. Diffusers must be located so that the bubbles stay in the water column as long as possible. Once a bubble has risen to the water surface oxygen transfer stops and its oxygen is lost. To maximize contact time diffusers are often located in deep areas of the pond or in pipelines. Low oxygen transfer efficiencies (40 to 50%) are the main disadvantage of this system.

An array of eight ceramic diffusers (2 ft long) has been fitted around the inside of a 24-in diameter pipeline located at the Quinsam Hatchery on Vancouver Island. Oxygen is introduced at the head of the pipeline and fine bubbles are swept along its 40 ft length. At a water flow of 5 cfs the oxygen transfer efficiency is 50% — nitrogen is also removed. The diffusers are supplied with pure oxygen stored in a liquid bulk tank. This system consists of an oxygen source, supply lines and diffusers and so is highly portable and flexible.

2) *Low pressure sealed columns*. If a packed column is isolated from the atmosphere and supplied with pure oxygen, the rates of oxygen addition and nitrogen removal are increased. Two simple ways of isolating the column are shown in Figure 10

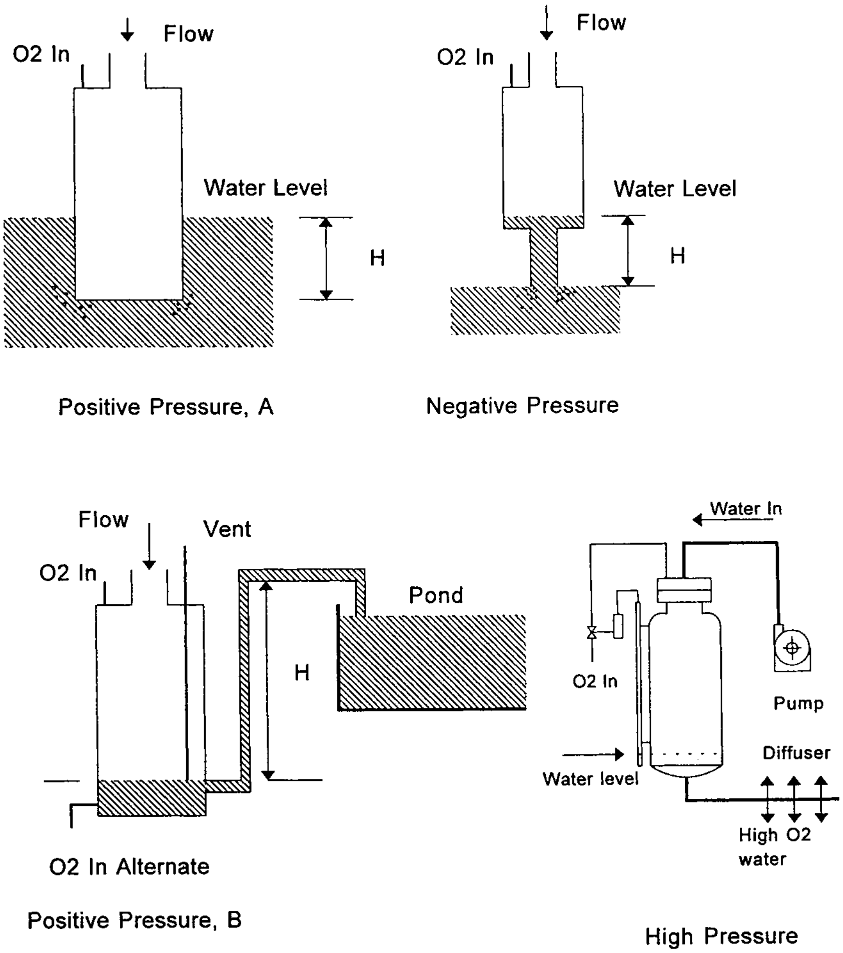

FIGURE 10. Schematic diagrams of pressurized packed columns for addition or removal of dissolved gas.

(Barratt 1989). In the first case the column has been sealed at the top and submerged to a water depth H. The total gas pressure in the column is 760 + H; if H = 0.5 m the pressure is 760 + 0.5*73.4 = 760 + 37 = 797 mmHg. In a static column the gas phase would be pure oxygen and its partial pressure would be 797 mmHg. In practice nitrogen is removed as water flows through the column and so some of this pressure is due to nitrogen. Excess gas would be vented out the bottom of the column. Bubbles consist of excess oxygen and nitrogen that have been stripped from the water. After leaving the column these bubbles must be given the opportunity to rise to the water surface. If they were swept down a pipeline, the nitrogen that had just been stripped could be reabsorbed.

The second column in Figure 10 uses the height of the outflow pipe to generate column pressure (760 + H + line losses). These columns are often fitted with a ventthat runs from the water level inside to the outside of the column. As nitrogen is removed and gas pressure builds up, the water level drops and gas is vented.

Low pressure columns are fairly efficient (70% to 85% oxygen transfer) and can remove nitrogen to below saturation. Mean inflow and outflow values for a low pressure column tested at Puntledge Hatchery over the summer of 1987 (mean Temp = 17.7°C) are summarized in Table 7.

3) *Negative pressure sealed columns.* This column is particularly effective at removing excess gas (Boersen and Chesney 1987). In this case the packed column is completely sealed and fitted with a submerged tail-pipe (Figure 10) (Barratt 1989). The diameter of the tail-pipe must match the flow requirements of the column. If it is too large the tail-pipe will not fill and if it is too small the column will flood. The column pressure is determined by the length of the tail-pipe: 760 - H. If H is 0.75 m the column pressure will be 760 - 55 = 705 mmHg. If oxygen is not added, the column will strip both oxygen and nitrogen. The length of the column, water flow rate, and the efficiency of the packing determine the final oxygen and nitrogen levels but in theory they could be reduced to 93% of saturation with H = 0.75 m. Stripped gas would be vented in the form of bubbles at the tail-pipe. Bubbles must be given the chance to come to the surface of the head tank and escape. If pure oxygen is added to the gas phase, nitrogen removal would be enhanced and dissolved oxygen would increase. These columns have oxygen transfer efficiencies of 40 to 50% (Boersen and Chesney 1987; Barratt 1989).

4) *High pressure sealed columns.* High pressure systems are used to treat a small sidestream flow which subsequently is mixed back into the main flow. These systems transfer massive amounts of oxygen at absorption efficiencies of at least 90%. A typical high pressure column (Figure 10) operates at an oxygen pressure of 50 psi or 2589 mmHg (Barratt 1989). Treated water has an oxygen concentration of 150 to 200 mg/l and is highly unstable after leaving the column. Slight pressure drops induce this stream to effervesce and lose oxygen. Diffuser structures must be carefully designed to mix the sidestream back into the main flow (Barratt 1989).

A very compact column, 38.1 cm (15 in) diameter by 183 cm (6 ft) high, is capable of oxygenating very high flows. At a temperature of 8°C, the sidestream flow of 575 l/min is increased from 11.8 mg/l to 185 mg/l. The column delivers oxygen to the diffusers at a rate of (185 - 11.8)*575 = 99,590 mg/min = 5.98 kg/h. If the diffusers operate at 90% efficiency, the column can deliver oxygen at a rate of 5.38 kg/h and raise the dissolved oxygen of a stream flowing at 34,000 l/min (20 cfs) from 4.0 mg/l to 6.6 mg/l. This

TABLE 7

Average oxygen and nitrogen levels at the inflow and outflow of a low pressure packed column tested at Puntledge Hatchery, B.C. in 1987.

| | **Inflow** | **Outflow** |
|---|---|---|
| Oxygen % | 101.7 | 183.9 |
| Oxygen mg/l | 9.6 | 17.5 |
| Nitrogen % | 104.7 | 88.6 |
| TGP % | 103.9 | 108.5 |

technique can be used during an emergency when flow reduction has occurred and large amounts of oxygen are required to keep the fish from suffocating.

It should be noted that there is no venting of the column so nitrogen stripping does not occur. Also the sidestream flow is usually small compared to the mainflow so background nitrogen levels can lead to high levels of total gas pressure. For example, if the dissolved oxygen in a pond is raised to 140% by side-stream injection and the background nitrogen is 109% then the TGP level is 115.5% and the risk of gas bubble trauma is high (Figure 11A).

### *Implications of Pond Design*

The oxygen levels experienced by fish depend on whether the pond has a circulating (mixed flow) or raceway (plug flow) design. In an ideal raceway the flow of water through the vessel is orderly with elements moving from inflow to outflow. If the fish are uniformly distributed, the oxygen concentration decreases down the length of the pond. In circulating ponds the inflow water is uniformly mixed almost immediately; the oxygen concentration is the same throughout the pond and there is no gradient (*see* above).

If oxygen of the inflow water is increased to 200% (22.5 mg/l) and ponds are loaded so that respiration consumes 14.5 mg/l, then the concentration in a raceway will decrease uniformly from 22.5 mg/l at the inflow to 8 mg/l at the outflow. In contrast, a circulating pond will have a uniform 8 mg/l over its entire volume (Westers and Pratt 1977).

This is an important consideration in oxygen supplementation because often the oxygen is added to air-saturated water without any nitrogen stripping. This means that the fish at the head of the raceway (at 10°C, sea level) would be exposed to a total gas pressure of 121% ($N_2$ = 100%, $O_2$ = 200%) while total gas pressure in the circulating pond would be 94% ($N_2$ = 100%, $O_2$ = 71%).

To counter the high TGP and extreme gradient, oxygen can be added at several points down the length of the raceway. Although plumbing is more complex, oxygen is distributed more evenly to the fish. The extreme gradient is alleviated and high TGPs at the pond inflow do not occur.

If several ponds are operated in series, it is convenient to add oxygen to the inflow of each pond. With this configuration there is also the opportunity of removing accumulated carbon dioxide, suspended solids and ammonia. As will be seen in the numerical examples presented in the next section, removing metabolites between ponds greatly increases the benefits of supplemental oxygen.

### *Limitations of $O_2$ Supplementation and the Need for Re-use*

A computer model was used to explore the trade-off in environmental quality that results when load rates are increased using oxygen supplementation. The potential to increase carrying capacity is often severely limited by site specific factors. Computer models do not give precise values but show trends and can give preliminary estimates of the optimum level of oxygen supplementation. Relationships used for these estimates are described in the previous section. The limits that determine carrying capacity were also discussed previously and are summarized as follows: dissolved oxygen, Table 4 (minimum of 6.4 mg/l used in examples to follow); un-ionized ammonia-N, maximum 12 $\mu$g/l; carbon dioxide, maximum 20 mg/l; suspended solids, maximum 15 mg/l; pH range 6.5 to 8.5.

Simulations are performed at the following conditions: BP = 760 mmHg (sea level); water temperature = 10°C; fish size = 10 g; and ration is at a maximum = 2%/d. Units for alkalinity are mg/l as $CaCO_3$ and ration level is expressed in g dry food per 100 g fish per day or %/d. The average daily oxygen consumption rate is 283 $mg \cdot kg^{-1} \cdot h^{-1}$ and the safe Ro is $1.37 * 283 = 387$ $mg \cdot kg^{-1} \cdot h^{-1}$. This value is used to predict the minimum daily oxygen concentration. The inflow ammonia-N and suspended solids levels are 0 mg/l while $CO_2$ is 0.8 mg/l (air-saturated water). $CO_2$, pH, ammonia and suspended solids concentrations refer to average daily values at the outflow of the pond.

*Effect of Alkalinity on Level of Oxygen Supplementation.* In this simulation, oxygen at the inflow of a rearing pond is increased to 200% of saturation (22.5 mg/l) and the background alkalinity of the water supply is low at 12 mg/l.

As loading rate increases water quality deteriorates — oxygen, carbon dioxide, total ammonia and suspended solids concentrations at the outflow of the pond increase with Lr (Figure 11B). pH drops as carbon dioxide increases and un-ionized ammonia levels off quickly as pH falls. The oxygen criterion (6.4 mg/l) is satisfied up to a load rate of 2.50 kg per l/min. However, at this loading, carbon dioxide and suspended solids are 15 and 17 mg/l respectively and pH has dropped to 6.3. In this simplified example the effect of ammonia on pH has been neglected.

This loading could be tolerated for a short time but would not be acceptable for long-term rearing. From Figure 11B, carbon dioxide, pH and suspended solids become limiting at loading rates of 3.5, 1.5 and 2.2 kg per l/min respectively. Un-ionized ammonia reaches a maximum of 0.34 $\mu$g/l and so is never limiting. pH is the first limiting factor and fixes the maximum loading rate at 1.5 kg of fish per l/min. This occurs because the water supply has an alkalinity of only 12 mg/l and so is sensitive to carbon dioxide accumulations.

Operating with an inflow oxygen of 200% is wasteful. At a loading rate of 1.5 kg per l/min, an inflow oxygen level of only 143% of saturation (16.1 mg/l) is required to maintain 6.4 mg/l at the outflow. This is the optimum degree of oxygen supplementation because the carrying capacity of the water supply is maximized and all water quality criteria are satisfied.

If the background alkalinity is increased to 50 mg/l, the effects of carbon dioxide are buffered and pH values do not drop to 6.5. Instead, suspended solids becomes limiting at a load rate of 2.2 kg per l/min (Figure 11B). At this limit the optimum degree of oxygen supplementation is 183% (21 mg/l).

*Increase in Total Gas Pressure.* In this simulation oxygen is added without removal of nitrogen and the oxygenated stream is introduced to the inflow of a raceway. The effect of elevated TGP was predicted using $ET_{50}$, the estimated time (days) to 50% mortality (*see* page 415). As the level of oxygen supersaturation increases, the chance of gas bubble disease increases i.e., $ET_{50}$ drops (Figure 9B). If the water supply is even slightly supersaturated with nitrogen the chance of gas bubble disease is high. With nitrogen saturation of 103%, oxygen must be less than 133% while if nitrogen is 90% the safe level for oxygen supersaturation is 196%. Figure 11A shows maximum oxygen saturation levels as a function of background nitrogen. It is evident that nitrogen supersaturation imposes a severe limit on oxygen supplementation.

*Buildup of Ammonia with Ponds in Series.* In this simulation water is treated between each pond in an attempt to increase carrying capacity. Oxygen is increased to 200% at the inflow to each pond, carbon dioxide is stripped to air saturation level (0.8 mg/l) and suspended solids are removed. Fish culture parameters are the same as previously given alkalinity is 20 mg/l. It is assumed that fish are evenly distributed so the X axis corresponds to increasing loading rate Lr. Water treatment takes place at the inflow (Lr=0), at the outflow of pond 1 (Lr = 2.50), and at the outflow of pond 2 (Lr = 5.0). The final loading at the outflow of pond 3 is 7.5 kg per l/min.

pH drops over the length of a pond but is restored between ponds as $CO_2$ is removed from the water. However ammonia increases steadily and un-ionized ammonia reaches high levels at the upstream ends of pond 2 and pond 3 (Figure 11B). pH and un-ionized ammonia values shown in Figure 11B are underestimated because the effects of ammonia on pH have not been taken into account. Nevertheless, the simplified model points out where critical un-ionized ammonia concentrations occur. In spite of oxygen supplementation and water treatment between ponds, carrying capacity of the water supply is limited by un-ionized ammonia.

### *Water Re-use*

To increase carrying capacity of a water supply, metabolite buildup as well as oxygen depletion must be controlled. In the previous example (three ponds in series, Figure 11B) oxygenation and $CO_2$ and suspended solids removal increased carrying capacity until ammonia became limiting.

Many options are available to treat water and increase carrying capacity: aeration and oxygenation for dissolved oxygen addition, sedimentation and filtration for suspended solids removal, degassing for removal of nitrogen and carbon dioxide, biological filtration for ammonia removal and pH control (Liao and Mayo 1972; Colt and Orwicz 1991*a*, 1991*b*; and *see* page 438). Disinfection may also be necessary as pathogenic microorganisms accumulate.

*Metabolite Buildup as a Function of Re-use Rate.* In re-use systems, the metabolite concentrations fish are exposed to depend not only on the load rate Lr but also on the degree of re-use (R) and the efficiency of the treatment system used to recondition the re-use stream (E). R is the proportion of the flow re-used. For example if the total pond flow is 1000 l/min (Qt) and 300 l/min is being re-used (Qr) and the make-up flow (Qa) is 700 l/min, then R = Qr/Qt = 300/1000 = 0.3 or 30%. If R = 0, no flow is re-used (Qr = 0) and there is a single pass through the pond. If R = 1, all the water is being re-used and there is no make up flow (Qa = 0). Treatment of the re-used stream results in a reduction in metabolite concentration. If the concentration after a single pass is 10 mg/l (Cf) and 7 mg/l is removed by the treatment system, the removal efficiency E is 0.7 or 70% and the re-used stream has a concentration of 0.3 mg/l or Cf∗(1−E).

If the make-up water is clean (i.e., free of metabolites), the concentration after a single pass Cf is Lr $R_N/60$ (*see* Equation 11) where Lr = pond load rate (kg of fish per l/min) and $R_N$ is the metabolite production rate ($mg{\cdot}kg^{-1}{\cdot}h^{-1}$). Each pass through the pond results in a portion of the flow being treated, mixed with makeup water and reintroduced to the pond. Since treatment is never 100% efficient, the pond concentration gradually builds up until a steady state concentration Cr is reached. Cr at re-use rate R and treatment efficiency E is given by:

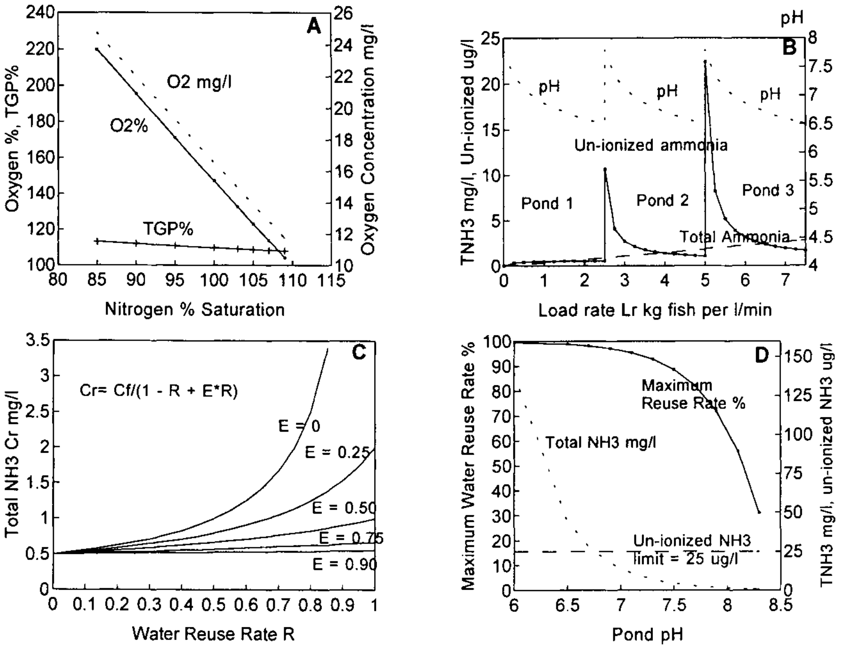

FIGURE 11. A. Safe oxygen levels as a function of background nitrogen level. The resulting total gas pressure is also shown. B. Changing total ammonia (mg/l), un-ionized ammonia (*u*g/l) and pH as water passes through 3 ponds in series. Each pond is loaded with fish at 2.5 kg per l/min. Carbon dioxide is removed between ponds so pH is restored. C. Total ammonia-N concentration (mg/l) as a function of water reuse rate (R) and removal efficiency (E). D. Maximum allowable water reuse rate vs pH. This simulation is based on an acceptable un-ionized ammonia-N concentration of 25 *u*g/l and assumes that other water quality parameters are maintained at acceptable levels.

$$Cr = Cf/(1 - R + R\,E) \text{ (Liao and Mayo 1972)} \qquad (20)$$

This relationship also applies to dissolved oxygen if the concept of deficit is used. For a single pass the deficit Df is the difference between the inflow and outflow: Df= Ci − Cf (assuming the inflow is near saturation). In a re-use system the steady state deficit Dr is given by: Dr = Df/(1 − R + R E). In this case treatment efficiency refers to the proportional decrease in oxygen deficit.

The buildup of total ammonia-N (un-ionized and ionized) in a pond illustrates how sensitive fish culture systems are to recycle rate R and treatment efficiency E. If a pond has a load rate of 1 kg per l/min and the excretion rate is $R_N$ =

30 $\mathrm{mg \cdot kg^{-1} \cdot h^{-1}}$ then the ammonia-N concentration after a single pass is Cf = 1*30/60 = 0.5 mg/l. The concentration at the pond outflow at different re-use rate and treatment efficiencies is shown in Figure 11C. If no treatment is performed (i.e., E = 0), the ammonia concentration at a 20% re-use rate is Cr = 0.63 mg/l (Cr = 0.5/(1 − 0.2)). At higher re-use rates the concentration increases quickly (Figure 11C).

*Constraints Posed by Ammonia Accumulation.* As noted in the previous discussion, many site specific factors (alkalinity, pond design, fish size, ration level, disease prevalence) determine whether oxygen, carbon dioxide, pH, ammonia or suspended solids are limiting. In many situations, ammonia is the most important limiting factor after oxygen (Westers and Pratt 1977; Haywood et al. 1980; Fivelstad 1988). High concentrations irritate gills and can precipitate bacterial gill disease. Ammonia accumulation is also responsible for increased levels of extremely toxic nitrite. High ammonia encourages the growth of nitrifying bacteria that oxidize ammonia to nitrite within the fish culture system. Large biological filters that convert ammonia to non-toxic nitrate (Burrows 1968; Speece 1973; Birkbeck and Walden 1976) or expensive ion exchange resins (Mercer et al. 1970; Bruin et al. 1981) are required for removal of ammonia (*see* page 438).

As shown in Figure 9A, the proportion of ammonia in the toxic (un-ionized) form increases with pH and temperature. Fivelstad (1988) proposed a maximum acceptable un-ionized ammonia-N concentration of 0.025 mg/l and has developed a model that predicts allowable re-use rates. These rates can be found by substituting Cr = 0.025 mg/l (25 $\mu$g/l) in the re-use equation (Cr = Cf/(1 − R + R E)) and solving for R. Since E = 0, R = 1 − Cf/Cr. Allowable re-use rates will be greatly reduced at high pH because the proportion of total ammonia in the un-ionized form is greater.

Consider a pond with 1000 kg of fish and a total flow of 1000 l/min (Lr = 1 kg per l/min) where the re-used portion is aerated so that the dissolved oxygen is restored to the inflow level of 11 mg/l but no ammonia is removed (E = 0). At typical oxygen consumption and ammonia production rates of Ro = 300 and $R_N$ = 30 $\mathrm{mg \cdot kg^{-1} \cdot h^{-1}}$, oxygen drops to 6 mg/l on a single pass and the ammonia-N increases to 0.5 mg/l. If the pH is 7.5 and temperature is 10°C then 0.58% of the ammonia is in the un-ionized form (Figure 9A) and the concentration after a single pass is Cf = .00584*500 = 2.92 $\mu$g/l. Since Cr = 25 $\mu$g/l the allowable re-use rate is R = 1 - 2.92/25 = 88%. With a pond biomass of 1000 kg the total flow of 1000 l/min consists of 880 l/min of re-use water and 120 l/min of make-up water. Allowable re-use rates for this pond are shown as a function of pH in Figure 11D.

*Empirical Approach to Determining Maximum Re-use Rate.* Haywood et al. (1980) used an empirical approach to determine the maximum allowable load rate. Ponds were loaded with coho salmon (97 g) at 1.5 kg per l/min and were subjected to a stepwise increase in re-use rate until R = 1 (100% re-use) was achieved. Re-use water was aerated and filtered for removal of suspended solids. Complex ammonia removal technology was not included and nitrogenous waste products were allowed to accumulate. Water was heated to a temperature of 15°C for maximum growth. Decrease in growth was used to indicate environmental degradation. The goal was to find the maximum re-use rate that could be attained using simple water treatment technology. This would allow heating costs of makeup water to be minimized.

Differences in growth between control and re-use ponds began to show up after 23 days at 80% re-use. It was concluded that re-use rates up to 70% did not cause reductions in growth. Mean levels of ammonia-N and urea-N over the 21 days at 70% re-use were 0.99 mg/l and 0.34 mg/l, respectively. Because the water was low in alkalinity, carbon dioxide from fish respiration depressed the pH to 6.64 and kept the un-ionized ammonia-N concentration low. The mean value over this period was only 0.0012 mg/l. At 95% re-use there was a lethal accumulation of nitrogenous waste products. The concentrations of nitrite-N, nitrate-N, ammonia-N and urea-N reached: 3.5, 22, 2.6 and 0.68 mg/l. Because of the low pH un-ionized ammonia was only 0.0036 mg/l. Toxicity occurred because of the extremely high level of nitrite.

## WATER RE-USE SYSTEMS

Reconditioning of effluent from fish tanks and returning this water to the fish is the final level of water re-use. Several aspects of water quality must be considered, including nitrogenous compounds, gases, and solids; ammonia must be oxidised to nitrate (or removed physically), oxygen must be replaced and $CO_2$ stripped, and solids (fish feces, feed fines and uneaten feed) must be removed. There are well established biological (bacterial) and chemical processes for reconditioning fish rearing water, but expense and difficulty increase exponentially with the degree of water re-use and with increases in biomass (density). For example, it is a relatively simple matter to recondition water at the 60% re-use level in a system with low fish biomass. It is much more challenging to re-use water at the 95% level (5% of total flow as new water) in trout tanks loaded at 70 $kg/m^3$ (*see* Rosenthal and Black 1993 for discussion of alternative definitions of percent re-use.) At high levels of re-use, disease may become a concern, and disinfection measures may also be required.

Re-use technology can benefit fish culture operations by reducing water costs where water is expensive (for example, deep well pumping costs) or severely limited in quantity (for example, desert climates). It is also possible to avoid heat loss where water is heated or chilled. Re-use systems also reduce the volume of effluent and make possible more efficient disposal or treatment of a concentrated effluent. However, these benefits must be carefully compared to the costs of re-use and also of risk entailed in maintaining a more complex system.

There are many types of re-use systems. These range from large central systems servicing an entire facility, to small stand-alone systems fitted to individual tanks. The most common and simplest system in use is the home aquarium's sub-gravel filter which achieves nearly 100% re-use levels with very small fish biomass. Larger public and research aquaria frequently combine high levels of re-use with very low fish densities; high standards of water quality are met in such systems, which are usually centralized (e.g., *see* Spotte 1970; Hawkins 1981). Re-use systems used for commercial food crop aquaculture such as trout farming are designed to handle much greater loads of metabolites and solids, and are usually correspondingly more expensive to operate, even though water quality standards may be below those of aquaria. Re-use systems for ocean ranching will lie in between the two extremes, with water quality standards set very high and densities lower than in commercial aquaculture.

Risk evaluation of re-use systems is complex and involves the operation of two systems: the fish tank and its life supporting structures, and the reconditioning system. In addition to increased opportunities for mechanical breakdown, the nature of the closed system or semi-closed system may allow disease challenge to increase (although external disease introductions may, in some cases, be reduced). Temperature in fish culture systems must be controlled. Although one benefit of water re-use is to conserve water temperature in warm or cold weather, re-use systems may lead to heating or cooling, especially if there is little make-up water entering the system. Insulating tanks or setting them in buildings or under roof cover may solve this problem. Back-up systems must be in place, as well as procedures for monitoring the entire system. Often overlooked in discussions of the benefits and costs of re-use technology is the labour factor. Some systems require enough additional maintenance labour to offset their advantages. In the discussions below, the main processes entailed in water re-use are discussed briefly, and some of the physical systems used to achieve water reconditioning goals are described.

### *Ammonia Removal*

Ammonia removal is a prime concern in recirculating systems. This usually is accomplished through bacterial oxidation of the toxic metabolic compound, ammonia first to nitrite, also toxic, and then to nitrate, which is essentially non-toxic to fish at levels likely to be found in re-use systems (Spotte 1970; Sigma 1983; and *see* page 411). The bacteria stated to be chiefly responsible for this activity are the autotrophs (chemolithotrophs), *Nitrosomonas*, and *Nitrobacter* spp. (e.g., *see* Wheaton et al. 1991). These organisms are ubiquitous soil organisms and will colonize any acceptable media, and their requirements are somewhat similar to those of salmonids; reasonably high levels of dissolved oxygen and nearly neutral pH, although they have a wider temperature range than salmonids. They function equally well in salt and fresh water, and it is probable that there are many species and strains within these genera. In comparison to many heterotrophic bacteria, these autotrophs grow slowly, and are associated with a slippery film which coats tank and filter media surfaces.

Biological nitrifying systems require a large surface area to support enough bacteria to effectively convert the ammonia produced by fish to nitrate; these systems are often known as biological filters or "biofilters," although they may not have any mechanical filtration function. There are many types of such filters described in the literature (e.g., *see* Wheaton 1977; Rogers and Klemetson 1985; Wheaton et al. 1991; and other papers cited below).

Although it seems clear that *Nitrosomonas* and *Nitrobacter* are the dominant nitrifiers in soils (Atlas and Bartha 1993) and probably in biofilters, the complete microbial community that develops in biofilters is complex and deserving of further study. It is possible that ammonia removal is accomplished by many genera and species, some autotrophs, and some heterotrophs using nitrogenous compounds for growth. In marine waters, there are many genera of nitrifying bacteria (e.g., *see* Austin 1988), and this is also true in fresh water (Atlas and Bartha 1993). As noted below, mature filters also acquire a complex community of invertebrates. This may add to the difficulty of quantifying filter performance. Acclimation of the nitrifying bacteria to changes in water quality (for example, temperature and pH) is often noted, but in some cases, this could be the replacement of one species or strain by another. Commercial bacteria concentrates are available to accelerate or improve filter performance, but these generally are not required and they may be ineffective (e.g., *see* Bower et al. 1981).

The chemical nitrifying reactions are the following.

1. *Nitrosomonas*:

$$NH_4^+ + 1.5\ O_2 = NO_2^- + 2H^+ + H_2O \tag{21}$$

2. *Nitrobacter*:

$$NO_2^- + 0.5O_2 = NO_3^- \tag{22}$$

It should be noted that the first reaction produces two hydrogen ions per ammonium compound oxidized; 7.4 mg of alkalinity is eliminated for each milligram of ammonium oxidized to nitrate, (Kaiser and Wheaton 1983). In closed systems with very high levels of re-use, pH can drop until at about 5.5, the nitrifying bacteria cease to function (they are not killed, however). Under such conditions, it is possible to see rising levels of ammonia, and sometimes nitrite if the populations of bacteria are differentially affected. Restoration of nearly neutral pH allow the bacteria to resume nitrification, and the system will regain target levels of ammonia and nitrite. This may take some time, however, and it is preferable to maintain pH if necessary with additions of a buffer source such as calcium carbonate.

Nitrate, the end product of nitrification, is non-toxic at levels usually encountered in re-use systems; the equilibrium levels of nitrate will be set by the amount of make-up water and nitrifying rate. Nitrate can be eliminated by passing water through plants that take up nitrate (MacKay and Van Toever 1981; Rakocy et al. 1993), although in commercial systems, this is usually not practical. Denitrification systems, which operate anaerobically, may also be used to eliminate nitrate (Rosenthal and Black 1993), but these are complicated and may be difficult to control (Whitson et al. 1993). Denitrification increases pH as noted below.

Each milligram of ammonium oxidized to nitrate requires about 4.2 mg of oxygen. Intermingled with the aerobic nitrifying bacteria are heterotrophic bacteria which make use of solid and dissolved organic matter entering the biofilter. The entire microbial system has a significant oxygen demand that must be met with an adequate oxygen delivery rate; a protection level only slightly lower than that of the fish must be maintained. Too great a loading of biological oxygen demand (BOD) may create anaerobic conditions in the biofilter, which will cause loss of nitrifying function until the necessary protection level of dissolved oxygen is restored. Anaerobic filter conditions will then result in high levels of ammonia in the fish tank; it has often been noted that filters under these conditions may produce ammonia. Nitrite levels may also increase under certain conditions of biofilter imbalance.

The examples of dissolved oxygen and pH problems illustrate the challenge of bacterially driven re-use systems; the culturist is dealing with two culture systems at all times, and perturbations in either system may cause persistent downstream disequilibria. The use of antibacterial agents for fish (antibiotics and bacteriostatins) may kill or de-activate the nitrifying bacteria, and abrupt increases in fish biomass may require some weeks of bacterial population increases in the filter to gain adequate nitrifying capacity. Such time lags may result in sub-standard water quality of enough magnitude and duration to cause growth depression or mortality in fish. Both fish and bacterial populations must be managed simultaneously.

An alternate means of ammonia removal is through the use of ion exchange media, usually in the form of zeolite columns, usually clinoptilolite (e.g., *see* Bruin et al. 1980; Smith et al. 1981). Most of these are naturally occurring clay minerals which act as ion exchange substrate, preferentially removing ammonia in exchange for cations such as sodium. When ion exchange columns are saturated with ammonia, they are flushed with a salt (NaCl) solution that shifts the equilibrium in favour of sodium; the ammonia is thus removed from the zeolite. Zeolite obviously cannot be used in saltwater systems. Ion exchange media also may have an effect on other ions (for example, removal of calcium and magnesium from water), and over time, may become clogged with dissolved and particulate organics (Watten and English 1985). The elimination of dependency on the complex bacterial populations in normal biofilters is the chief advantage of zeolite filters (*see* Wheaton 1977; Bruin et al. 1980; Watten and English 1985).

### *Types of Water Re-use Systems*

Re-use technology originated to some degree from sewage treatment technology which can be seen in many modern re-use systems. Much of the basic understanding of the water reconditioning processes in aquaculture, and the incorporation of this knowledge into operating systems, was attained in the 1970s and early 1980s (Wheaton 1977; Owsley 1993; Rosenthal and Black 1993).

Many systems are in use today, some constructed on site and others available in commercial units. While there is a large literature on re-use systems, many new designs are developed each year, and performance information often lags behind promotion. It is typical to note that a commercial system may rapidly evolve through many different versions, each with different characteristics. A good part of the available literature on commercial systems is found in non-refereed publications such as conference proceedings and trade journals. Literature in the 1970s adequately describes the basic processes and most of the systems used today (Owsley 1993; Rosenthal and Black 1993). More recent literature presents various combinations of previously described systems, some new technology and refinements in design, and is now approaching better quantification and improved modelling of the processes involved in recirculation (Malone et al. 1993), as well as whole systems (e.g., *see* Westerman et al. 1993).

Reviews of the basic types of systems in use can be found in Wheaton (1977), and Wheaton et al. (1991). The latter paper provides many useful references on this topic. Each function in the water re-use process can be achieved though different sub-systems, and these may be combined differently into complete systems. This, in part, explains why there are so many designs in use or offered for sale. In the following review, the systems used to support the various processes are discussed separately, followed by a few examples of complete re-use units.

*Water Transport.* Movement of water through treatment systems may be accomplished by pumps or by low-head airlift systems. The former allows more flexibility in flow rate and systems design, including aeration, but usually entails higher energy costs than airlift systems which can operate on high volume/low pressure air blowers. However, airlift systems must be carefully designed to achieve required flow rates, oxygenate water, and avoid gas supersaturation. It is clear that either system type is dependent on external power, and back-up systems must be included in the design.

*Biofilter Design.* Bacterial re-use systems (biofilters) may make use of activated sludge, but much more commonly utilize thin biofilms (sometimes known as fixed film) on a wide variety of materials whose main function is to provide a large surface area in a compact space. The enormous variety of substrate used (for example, sand, rock, beads, plastic saddles, bio-rings and telerets, tubes and battings) are chosen for their ability to support thin film bacterial populations without excessive sloughing, to provide high surface area and permeability to water flow, and to provide either resistance to clogging or ease of washing. Such systems can be roughly classified as having either submerged or non-submerged media.

Submerged systems employ up- or down-welling water flow, or in a few cases, horizontal flow. The simplest early submerged filters consisted of a gravel bed in a chamber through which water up-welled. Bacteria colonized gravel (or other material such as oyster shell), and solids were cleaned out periodically from a sump below the gravel bed and by manually washing gravel. Modifications included compressed air-assisted back-washing and use of more than one type of media. Such media beds may be "fluidized," that is, expanded by water velocity to increase permeability and create gentle motion within the bed which reduces clogging (e.g., *see* Wheaton 1977; Summerfelt and Cleasby 1993). As noted above, pressure sand filters filled with several densities of substrate may also be used, usually in an attempt to remove solids and nitrify ammonia in one operation. Pressure filters entail significant hydraulic head losses and require more powerful, and hence more expensive pumps than are required for open systems. Automated back-flushing usually is also required.

Many recent submerged filter designs utilize various types of plastic beads (usually fluidized), sinking with up-welling flow or floating with down-welling flow. Beads provide a very large surface area, and they may be textured to increase this further and to reduce sloughing of the bacterial film (Malone et al. 1993). The motion of beads in the column helps to reduce clogging, but all such filters will eventually clog with particulate matter and so, must be cleaned frequently. Cleaning operations often strip away too large a proportion of nitrifying bacteria and may also be labour-intensive. Much of filter design is aimed at solving these two problems.

Non-submerged biofilters may take the forms of trickle filters, rotating drums, rotating disks, and other designs, having in common exposure of the media and its thin film of bacteria to the air. This ensures that the bacteria will always have adequate dissolved oxygen levels. In the case of trickle filters (usually containers of plastic media through which water splashes in a continuous film), system aeration and nitrification are combined in one operation with no danger of gas supersaturation. Such filters may become clogged, but can be cleaned relatively easily (Rosenthal 1980; White 1990). Rotating disks or drums may be operated with airlift systems, or electric motors, and they can be self-cleaning.

Filter cleaning requires washing with water, and the amount of water used and sent to waste may become a significant component of a system's overall operating costs (e.g., *see* Libey 1993).

*Solids Removal.* In all re-use systems, solids must be removed either continuously or periodically, and this generally is much more challenging than the other functions in re-use systems; in most high-density fish culture re-use systems, solids removal is probably the chief limiting factor. Very small particulates are difficult to settle out of moving water and pressure filtration, although effective, is expensive both in terms of capital and operating costs. If solids are allowed to build up in the biofilter portion of the system, nitrifying bacteria will be out-competed by various heterotrophs. Rogers and Klemetson (1985) noted that when BOD levels exceed 30 mg/l, nitrification will cease, presumably due to lowered oxygen levels and physical over-layering of nitrifying bacteria by faster-growing heterotrophs; other studies suggest that still lower levels of BOD inhibit nitrification (Lu and Piedrahita 1993; Rosenthal and Black 1993). However, this will be dependent on filter design.

The swimming pool sand filter (rapid sand filter) is a typical pressure filter which can remove particles as small as 30 $\mu$m, depending on sand grain size. Such filters must be back-flushed frequently, and entail large losses of hydraulic head and, as noted above, require powerful pumps for their operation. More commonly, particulate is removed by a variety of settling systems, which attempt to reduce turbulence and flow velocities below critical settling velocities of the particles to be removed. Some of these systems are effective, but solids must be moved gently to avoid break-up into small, low-density particles which will not settle out. Fish feces may be easily settled out of water, unless they are mechanically broken into small bits which renders them very difficult to remove. Double stand-pipes may be used to separate feces, as they first fall to the bottom of the rearing tank; a side stream of water with concentrations of feces is shunted to waste. Other strategies may be used to remove feces and large particles before water is oxygenated or passed through biofilters. At high re-use rates, dissolved organic compounds may become a problem; their removal is discussed below.

Settling systems may take the form of large rectangular tanks (Wheaton 1977), cones (sometimes using swirl separation) with drains at the bottom, or tubes creating low velocity zones (White 1990; Libey 1993). In general, the smaller (and lighter) the particle, the more difficult settling becomes so that small, low-density and flocculent matter is nearly impossible to remove by gravity. Failure to remove particulate not only has a negative effect on the biofilter, but also may affect fish health through damaged gills and elevated suspended bacterial loads. The nature of suspended and settleable solids, as well as solids removal systems, is reviewed by Wheaton (1977); Lawson (1987); Cripps (1993); Goldsmith and Wang (1993); and Weston et al. in Chapter 16 and *see* Figure 12.

The heterotroph population in filters will consume some fine particulate, and there is usually a well-developed but not often described community of protozoans and small multicellular invertebrates [for example, rotifers, nematodes, and others in biofilters (Martin 1968)]. These will also interact with fine particulate and associated bacteria, and will add to the BOD of the filter system.

*Dissolved Organic Compounds*. Dissolved organics will build up from leaching of fish feces, uneaten food, and other products produced by fish (for example, mucus), and from the filter, as partially utilized compounds. At high fish density and high re-use

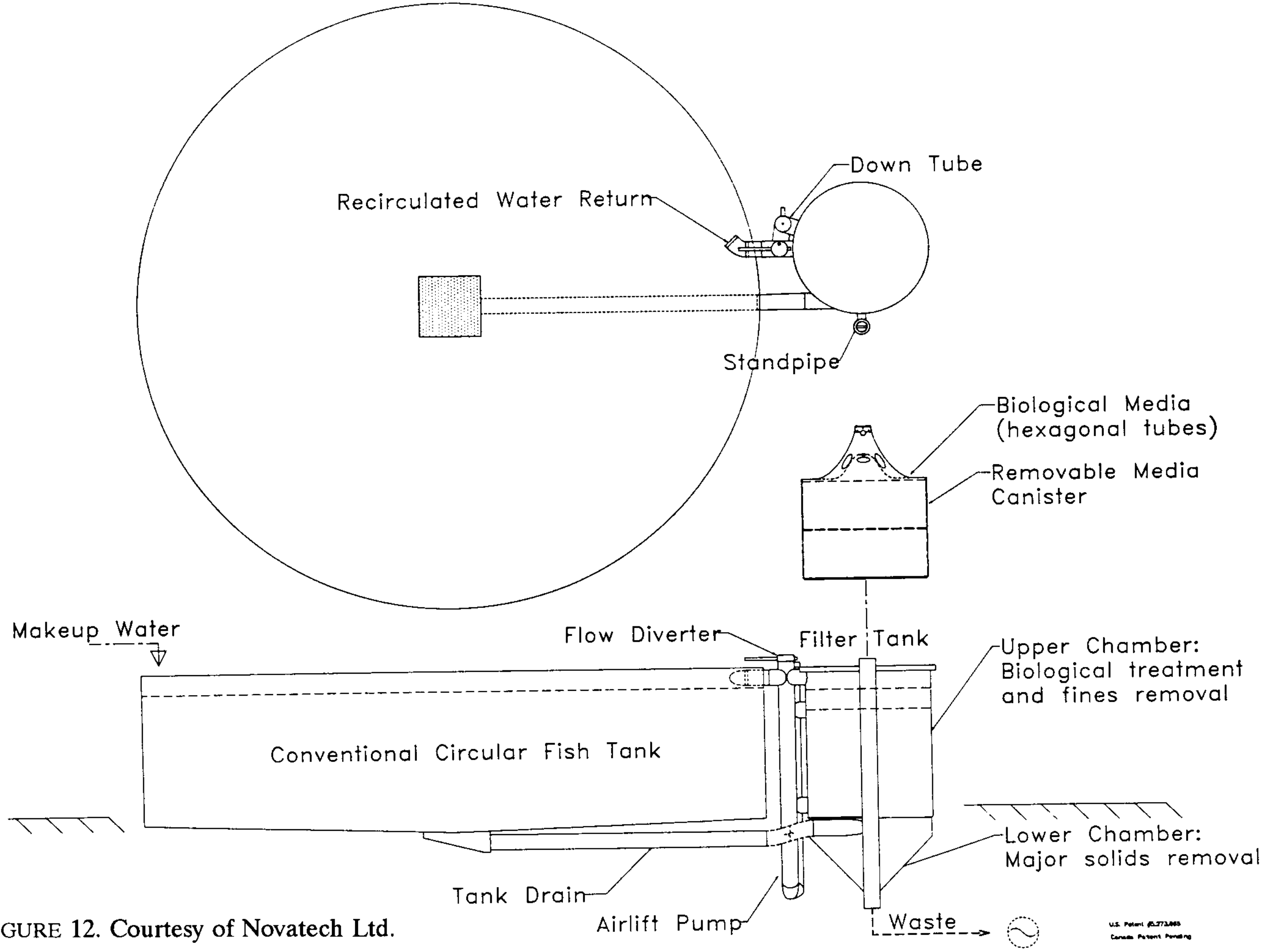
Down Tube
Recirculated Water Return
Standpipe
Biological Media (hexagonal tubes)
Removable Media Canister
Makeup Water
Flow Diverter
Filter Tank
Upper Chamber: Biological treatment and fines removal
Conventional Circular Fish Tank
Lower Chamber: Major solids removal
Tank Drain
Airlift Pump
Waste
U.S. Patent
Canada Patent Pending

FIGURE 12. Courtesy of Novatech Ltd.

rates, this material can build up and discolour water. There is an oxygen demand associated with these organics, and they may provide a substrate for bacteria (although many compounds are refractory, which is why they accumulate). In some cases, off-flavour in fish develops in re-use systems, probably caused by compounds produced by certain heterotrophic bacteria. This can be a serious marketing problem.

Dissolved organics can be controlled by periodic water changes, increasing the proportion of make-up water (new water), or reducing fish density or feed rates. However, these measures may defeat benefits of re-using water. Ozone may be used to break organic molecules into smaller, less refractory compounds which can be eliminated by heterotrophic bacteria in the system (Rosenthal 1980; Rosenthal and Black 1993). Foam-fractionating systems remove dissolved organics and very fine particles through bubbling air in a chamber, creating a foam of surface active organics which is then removed from the system. There are many foam fractionating designs (Wheaton 1977; Wheaton et al. 1979). Another way of removing dissolved organics is through activated carbon beds; these are effective, but are subject to rapid clogging unless water is pre-filtered. Activated carbon is expensive and must be replaced frequently, and may alter water chemistry.

*Gas Exchange*. Gas exchange in re-use systems is done through aeration (*see* page 428); water may be pumped into an elevated aeration tower, in which case proper design of the aeration system will ensure that dissolved gases are adjusted to meet both fish health standards and the requirements of bacteria. Although gas exchange may take place at any point in the system, $CO_2$ and dissolved oxygen concentrations are altered as water passes through both fish and bacteria segments of the system. In cases where air is introduced by an airlift system, which is also moving water through the system, it is critical to achieve adequate gas exchange at the required flows, since flow and aeration are coupled. Usually, $CO_2$ stripping and re-oxygenation take place simultaneously. As long as gas supersaturation is not introduced by pressure systems, and if oxygen levels are brought up to 95% saturation and $CO_2$ kept at very low levels, then dissolved gases will not pose problems ($CO_2$ levels must be below 15-20 ppm in the rearing water —*see* page 411).

Air diffusers may be used in the culture tank, but these require high pressure air pumps, and may break up particulate matter and alter water flow patterns in the tank; they may not adequately strip $CO_2$ from culture water. If placed deeply in a tank, they may contribute to gas super-saturation. Water may be pumped to a gravity splash aerator and, although this entails the expense of a mechanical pump, gas exchange is easily accomplished. As noted, air lift systems may move water and exchange gases in one operation, and pure oxygen can be added to the system as described on page 425. Although oxygen is used in some high-density re-use systems, it is uncommon because of expense, and because the process of moving enough water through the biofilter to achieve nitrification generally creates adequate opportunity to exchange gases, at least for moderate fish densities. In very high performance systems with high fish density, oxygen supplementation may become an option (Van Gorder 1991).

Since nitrifying bacteria require somewhat lower protection levels of dissolved oxygen than salmonids (2-4 mg/l), it is possible to adjust dissolved gases after water passes through the biofilter, and before it enters the fish-rearing chamber, as long as the BOD of the filter does not reduce DO levels below those required for good filter performance. Two milligrams per litre is often given as the minimum level of DO to support nitrifying activity (e.g., Spotte 1970; Wheaton 1977), but there is some evidence that higher levels of DO (ca 4 mg/l) will improve nitrifying activity (Kaiser and Wheaton 1983). As an example, suppose the following: reaerated water in a fish tank has a dissolved oxygen protection level of 7 mg/l. This water passes from the fish tank into a biofilter with a DO of 7 mg/l; the total oxygen demand of bacteria and associated organisms is 3 mg/l of flow so that no portion of the biofilter receives less than 4 mg/l of DO. The water then moves into the reaeration-gas exchange system and re-enters the fish tank. DO protection levels of both systems are met.

*Biofilter Capacity.* Although the above example deals with oxygen as a limiting factor to nitrification, ammonia concentration, biofilm thickness, and water retention time in the biofilter are all interacting variables which affect and may also limit nitrification (Wheaton et al. 1991; Lu and Piedrahita 1993). Determining the correct size of filter for a given application (or determining the fish loading permitted for a specified filter) is therefore difficult because there are many interacting variables. The biomass of nitrifying bacteria is a major variable; the more bacteria per unit-filter (or per unit-flow), the more ammonia will be nitrified per unit of time. This leads to concern over surface area within the filter, and most designs aim at maximizing surface area. It is important to note that the effective ammonia concentration available to bacteria may be best expressed as the mass loading, the concentration multiplied by the flow rate. If there is adequate surface area (large bacterial population), then moving water through the filter more rapidly delivers more ammonia to the bacteria per unit of time. In this way, relatively small filters with short water residence times have been designed, which nitrify at very low ammonia concentrations (Paller 1992). Increased flow of water through biofilters may be achieved independently of flow through the fish container, through a side stream circulating water through the filter.

Thickness of biofilm is also a factor in nitrification efficiency; thin films are more efficient than thick films, since inner layers of bacteria will not be easily exposed to ammonium and nitrite in the passing water. As bacteria grow, the film thickens until inner layers die and patches of film slough away. If sloughing of the biofilm during cleaning is excessive, then the growth rate of bacteria can become rate-limiting, and ammonia or nitrite levels may rise temporarily in the system. The media and its handling, as well as water velocity, influence biofilm thickness and sloughing rate, but this is very difficult to quantify (Wheaton et al. 1991).

Since both bacterial growth (replacement) and nitrifying activity are affected by ammonia (and nitrite) levels, the very low levels of these metabolites required for fish health (*see* page 411) imply that ammonia concentration will usually limit the rate of nitrification in biofilters. This generally is true at total ammonia concentrations less than 2 mg/l. The efficiency of ammonia removal in fish culture biofilters is one to two orders of magnitude less than that found in sewage treatment plants (Kaiser and Wheaton 1983).

Growth rates of nitrifying bacteria are affected by temperature and ammonia concentrations (assuming factors such as oxygen and pH are not limiting). New filters must be conditioned before use; that is, they must be operated in the presence of ammonia for several weeks until populations of bacteria have built up and are capable of handling the ammonia to be produced by fish in the rearing container. Conditioning time can be shortened if both ammonia and nitrite are added to a system at elevated levels without fish (Manthe 1991); once filter media is conditioned, it can be maintained in a nutrient-rich system without fish or dried in air as a standby.

Temperature affects the rate of nitrification so that, at low temperatures, nitrification rate becomes reduced, although fish metabolism and feed rates are also reduced at low temperatures, which reduces ammonia production. Some recent trials indicate that filters may, in fact, operate quite effectively at low temperatures (T.B. White, Nova Tech Ltd., Nanaimo, B.C., Canada, pers. comm.). It is clear that variables in nitrification are numerous, and while it is possible to model nitrification performance in a filter (e.g., Speece 1973), in practice, empirical data from trials are required to assess filter performance under production conditions. Most commercial systems exceed required nitrification performance at normal ammonia loadings, if they are operated properly. They are more likely to encounter problems with other factors such as particulates and dissolved organics.

It was noted above that bacterial biofilters require time to become fully colonized and then need careful maintenance to remain effective. Sudden changes in fish biomass or ration may easily overload the biofilter's capacity to nitrify ammonia. This is sometimes known as "load-shock" (Manthe 1991; Malone et al. 1993). Consequently, fish production and stocking rates in tanks must be planned with the microbial communities in mind.

*pH Control.* In systems with a high degree of re-use, the $H^+$ ions produced in the first nitrification reaction will steadily lower pH to an equilibrium level set by the rate of H+ ion production (controlled by fish biomass and feeding rate) and the amount of new water entering the system. $CO_2$ produced by fish and by the microbial community in the filter will also reduce pH, although bacteria may use some $CO_2$ as a carbon source. It is assumed that most $CO_2$ is eliminated at the point of gas exchange. As pH falls below 7, nitrifying bacteria are negatively affected, with *Nitrosomonas* (bacteria which oxidize ammonium ion) more strongly affected than *Nitrobacter*, which has a lower pH optimum (Kaiser and Wheaton 1983; Manthe 1991). Consequently, as pH falls, both ammonium and nitrite concentrations rise in the system's water, but there may be changes in the proportion of ammonia to nitrite that are difficult to predict (Lawson and Wheaton 1983).

There is evidence that nitrifying bacteria can acclimate to lower pH and regain full nitrifying rates at pH levels of about 6. The effect of lowered pH is considered to be inhibitory rather than toxic, since bacterial colonies quickly regain full nitrifying ability when pH is restored to the optimum level (Kaiser and Wheaton 1983). The simplest means of controlling pH is through the addition of a medium composed of limestone or shell, although this has limitations (Spotte 1970; Bower et al. 1981, for seawater applications), and it may not be convenient in many modern filter designs. The addition of a dissolved buffer is also effective and simple. Otte and Rosenthal (1979) suggest that a denitrifying column to eliminate nitrate ($NO_3$) also helped to maintain neutral pH levels. It has also been suggested that sequential biofilters could allow the two genera of bacteria to exist in pH regimes closer to their optima (*Nitrosomonas* first producing lowered pH for the next filter in which *Nitrobacter* would predominate). However, this is generally not done in commercial systems.

### *Disinfection and Problems with Fish Disease Management*

Disease organisms can theoretically multiply in re-use systems that are maintained intentionally as bacteria-rich systems, through which water is recycled. In some cases, it may be desirable to disinfect water in the system to reduce total suspended pathogen loadings. Treating with chemical substances to reduce bacteria would obviously be a risk to the filter, so that such treatments of fish must usually be done in isolation from the biofilter. Water can be disinfected by ultraviolet light (Rodriguez 1993), but this requires that particulate be removed first; otherwise, bacteria are shielded by particles. Such filtration may be expensive, as are UV light units which must be replaced annually.

Ozonation may be a better approach (Rosenthal 1980; Rosenthal and Black 1993). Ozone not only disinfects, but, as noted above, it is reported to break down refractory dissolved organic compounds, rendering them more easily consumed by heterotrophs. The various species of oxygen compounds produced by ozonation must be eliminated from water before it is returned to biofilter or fish; intermittent ozonation may be effective in recirculation systems (Rosenthal and Black 1993). Fish disease prevention through water quality and stress management is clearly a high priority in re-use systems, but because biofilters entail the culture of large bacterial populations, system disinfection is not desirable or possible. Maintaining a balanced microbial population may reduce the chances of a pathogen outbreak in the system (Lewis et al. 1987).

### *Conclusion*

Water re-use technology for aquaculture has now been developed to make possible consistent commercial applications, but it is not without expense and risk. Cost savings in water supply, temperature control, and effluent treatment must be weighed against power and labour costs to operate the re-use system (e.g., Gempesaw et al. 1993). While power costs may be readily calculated, labour costs may be very difficult to estimate. Filters must be cleaned frequently, and air and water pumps maintained on a regular schedule. Often, components must be replaced. Water quality monitoring in re-use systems requires much more effort than in single-pass systems, and data must be recorded and managed (White 1990).

Risk is difficult to assess. While all hatchery systems are prey to water interruption events, the re-use system is much more complex and subject to failure at several points. This can include biological problems with filters, power supply to water and air pumps, and difficulty in dealing with fish disease or parasite outbreaks. Backup systems must be in place.

On the positive side, re-use technology may pay for itself very quickly under some conditions, and well-developed systems can free aquaculture from the usual site limitations. For example, crops of fish may be reared very close to major markets, thereby reducing transportation costs and increasing profits. Re-use systems inherently require more control over the environment of fish, and environmental control may offer much increased and more reliable production. As environmental concerns over effluent increase, re-use systems may offer better and less expensive control of nutrient releases (*see* Rosenthal et al. 1993).

## AREAS REQUIRING FURTHER RESEARCH

The toxicity of ammonia is not clear and suggested safe concentrations of un-ionized ammonia vary widely. More study of the chemical environment at the gill surface may help resolve some of this confusion. The effect of exercise on fish growth, conversion efficiency requires more study. Existing work needs to be applied to other species of salmonids and mechanisms underlying exercise-growth interactions should be clarified. It is especially important to apply existing studies to survival of released fish. For example a small increase in ocean survival in a sea-ranching operation could be worth millions of dollars. Studies of fish behaviour, which have been made mainly in an ecological context, should be applied to the hatchery setting. This could lead to pronounced reductions in stress, better growth performance, and higher quality of fish produced. Work in this area may also lead to increases in safe density levels which would reduce fish production costs. Although much is known about water quality requirements of salmonids, the effect of suspended particles on gill tissue has not been adequately studied or quantified.

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## Chapter 7

# GROWTH OF SALMONIDS

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## INTRODUCTION

The primary purpose of this chapter is to present available data on salmonid growth, and to apply a simple algorithm by which comparisons among various data sets of fish growth can be made. This paper also reviews how various intrinsic and extrinsic factors can affect fish growth. Other than a few introductory comments about the nature of fish growth and how it is measured, relatively little attention will be paid to the more theoretical aspects of fish growth. Recent reviews by Ricker (1979), Weatherley and Gill (1987), and Busacker et al. (1990) provide a comprehensive coverage of the more theoretical aspects of this broad subject. It is intended that the quantitative tool used in comparing the various data sets in this chapter might be of use to aquaculturists in planning production, and in the comparison of growth data from different sources.

Growth is one component of the energy budget of the fish. In the most basic terms, energy flowing into the animal as food is used to maintain the essential metabolic processes, and then is either assimilated into tissues or eliminated. The assimilation of the consumed energy can be in the form of tissue elaboration or the production of reproductive products. In addition to these anabolic processes, energy is also required for physical activity and for the consumption, digestion, and absorption of food, also known as specific dynamic action. The production of heat as a product of metabolism, and the elimination of urine and fecal waste are outputs of the system. Such an energy budget can be summarized in the following equation:

$$Ef = Eg + Er + Esm + Esda + Ea + Eh + Ew + Eo \qquad (1)$$

where E is energy content of: the food consumed (f), growth (g) or net change in the energy content of the animal, reproductive products (r), standard metabolism (sm), specific dynamic action (sda), activity (a), heat (h), solid and urine waste (w), and other (o) components such as the energetic cost of stressful conditions, or the energetics of ionic and osmotic regulation. Detailed discussions about the bioenergetic budget can be found in Brett and Groves (1979), Elliot (1979), and Cho et al. (1982) (*see also* Chapter 2).

In this chapter, growth refers to the increase in the mass of the animal. As opposed to the concept of population growth, as being an increase in the number of a population, the increase in the mass of the individual and the consequent increase in the total biomass of the cultured organism is of central concern in aquaculture. Fish growth can be defined as an elaboration of tissue (*see* Chapter 2). Although, the increase in the mass of various tissues within the fish body reflects the nature of the increase in the mass of the whole animal, the common practice in aquaculture is the measurement of changes in body weight or length. As in many other groups of cultured fishes, the body weight of the fish is probably the most important parameter in terms of production. Government agencies that culture salmonids for the enhancement of natural stocks are concerned with the production of a target number of fish of acceptable body conformation, and the commercial aquaculturist is concerned with the total biomass of fish of a certain weight for sale.

Current information about growth in fishes was obtained from several sources for this review. Literature pertinent to fish growth was reviewed and, wherever possible, data were extracted for these analyses. Growth data were also obtained from salmon farms in British Columbia, as well as from research projects of graduate students in my laboratory. No attempt was made to discern statistical difference among data sets because of the differences in conditions that were specific to each study, or culture condition.

## QUANTITATIVE DESCRIPTIONS OF GROWTH

In the most basic terms, the determination of growth depends on accurate measurements of the mass (m) of the animal. Figures 1 - 3 show some of the general trends in growth which were found in the data. They confirm the classical trends shown by previously published evidence in this field. Growth can be defined as the change in body mass $(m2 - m1)$. A growth rate can be defined, then, as a change in body mass per unit time (t), or $(m2 - m1) / t$. Growth rates change as a function of body size, such that the highest growth rates are found in young, small animals. Growth rates, relative to body size, can be calculated by dividing the absolute growth rate above by the initial body size $[(m2 - m1) / t \times m1]$. If the increase in body mass is not linear, the weight measurements must be transformed in order to apply these linear equations. The natural

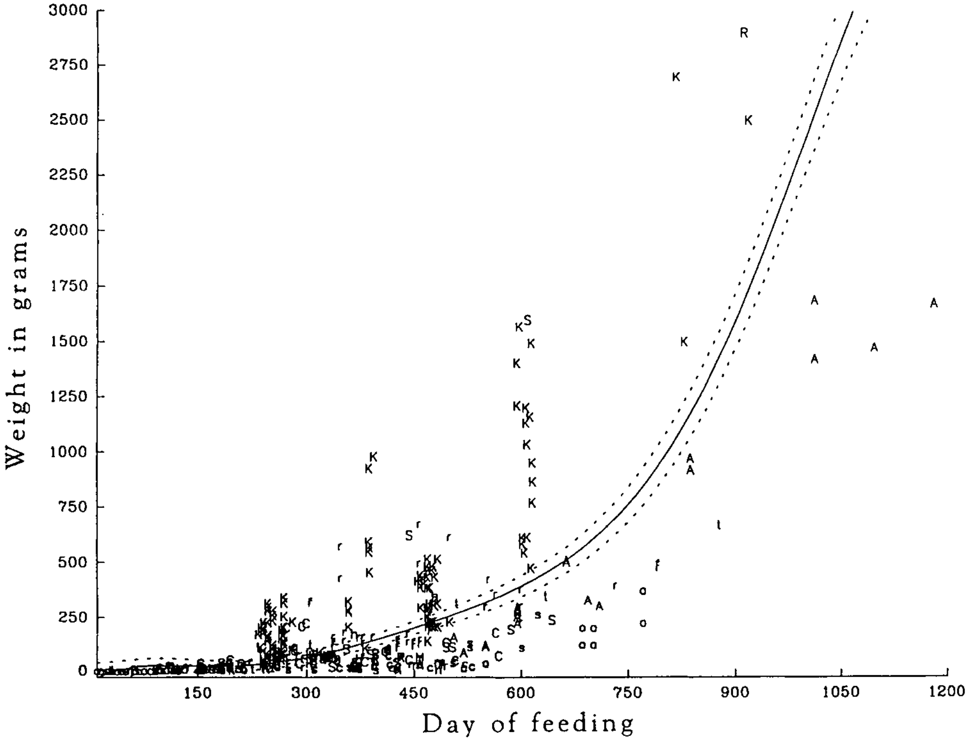

FIGURE 1. Wet weight (in grams) of various fish groups. Age of fish shown is the approximate number of days elapsed since commencement of feeding. Points are compiled from many studies (*see* references marked with an *) and show control or "normal" growing fish. The solid line shows the best fit regression line with its 95% confidence limits as dotted lines. Individual points on the plot are shown as letters with A,a representing *Salmo salar*, H,h for *Salvelinus alpinus*, T,t for other trout species, R,r for *Oncorhynchus mykiss*, K,k for *O. tshawytscha*, C,c for *O. kisutch*, M,m for *O. masou*, S,s for other salmon species. Lowercase letters indicate fish groups in fresh water while uppercase letters indicate those in salt water.

logarithm of weight usually accomplishes this. The calculated growth rates are then called specific growth rate and are described by the equation (ln m2 − ln m1) / t × ln m1.

Direct measurements of body mass and the calculation of growth can be done reliably and without killing the fish. While length is an important parameter to measure, weight measurements are usually the central measurement. Length almost always

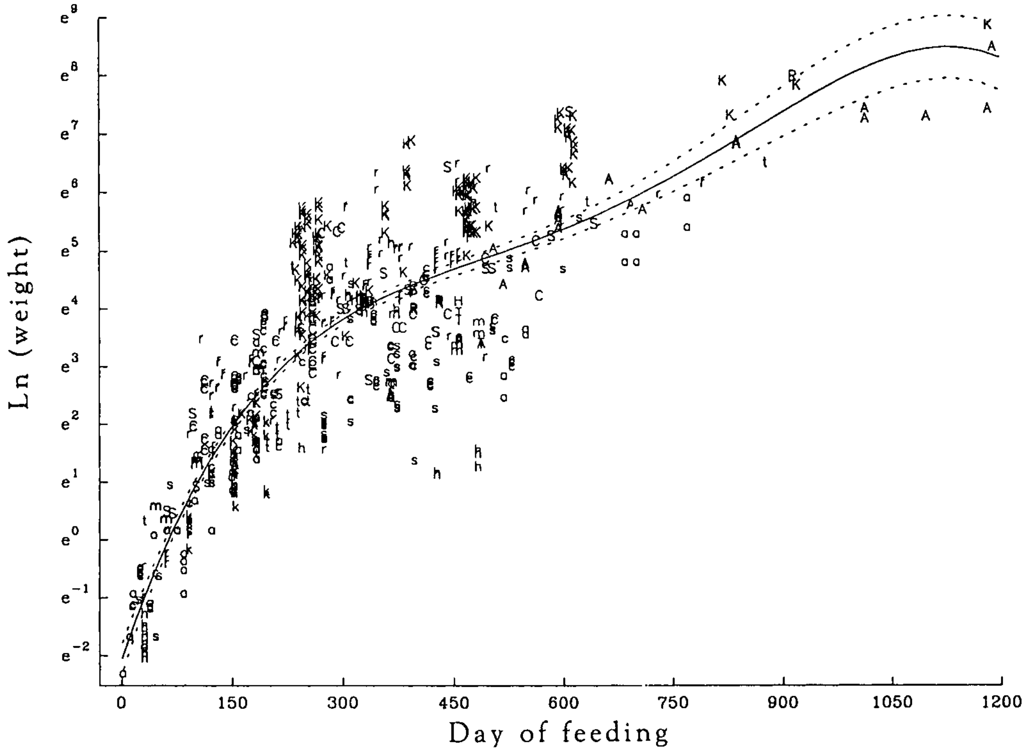

Figure 2. Natural log of wet weight (in grams) of various fish groups. Age of fish shown is the approximate number of days elapsed since commencement of feeding. Points are compiled from many studies (*see* references marked with an *) and show control or "normal" growing fish. The solid line shows the best fit regression line with its 95% confidence limits as dotted lines. Individual points on the plot are shown as letters with A,a representing *Salmo salar*, H,h for *Salvelinus alpinus*, T,t for other trout species, R,r for *Oncorhynchus mykiss*, K,k for *O. tshawytscha*, C,c for *O. kisutch*, M,m for *O. masou*, S,s for other salmon species. Lowercase letters indicate fish groups in fresh water while uppercase letters indicate those in salt water.

increases with time, whereas weight can increase or decrease with time, depending on the influences of factors that can affect the increase in tissue mass (discussed in more detail below). Both length and weight can be easily measured when individual fish are being handled. The measuring device, with a ruler incorporated into it, can be tared on the balance, and both measurements can be made simultaneously. Estimates of the average individual weight can also be calculated from the rough estimation of body weight from a sample taken from a population in a rearing container. Typically, a group of fish are netted out into a bucket containing some water, which has been tared, and after the total

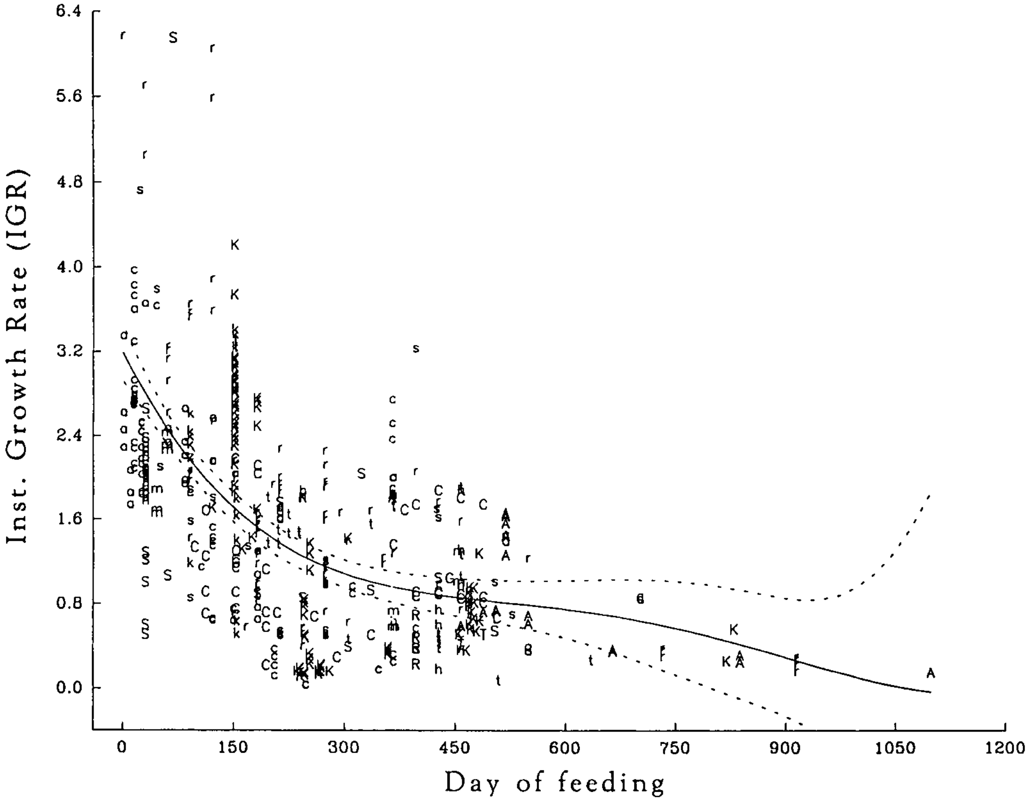

Figure 3. Instantaneous growth rate of various fish groups. Age of fish shown is the approximate number of days elapsed since commencement of feeding. Points are compiled from many studies (*see* references marked with an *) and show control or "normal" growing fish. The solid line shows the best fit regression line with its 95% confidence limits as dotted lines. Individual points on the plot are shown as letters with A,a representing *Salmo salar*, H,h for *Salvelinus alpinus*, T,t for other trout species, R,r for *Oncorhynchus mykiss*, K,k for *O. tshawytscha*, C,c for *O. kisutch*, M,m for *O. masou*, S,s for other salmon species. Lowercase letters indicate fish groups in fresh water while uppercase letters indicate those in salt water.

weight of the sample has been determined, the fish are counted back into the rearing container. The total weight is divided by the fish number to arrive at an estimate of the weight of the individuals in that population.

Other estimates of fish growth are based on indirect assessments of changes in physical attributes of the fish, which are related to one or more aspects of growth. Some of those include: the reading of annuli from the scales or other calcified tissues such as the otoliths; the uptake of glycine by scales *in vitro*; the determination of RNA:DNA ratios in white muscle; and the determination of protein synthesis. The reading of annuli from scales has provided fisheries biologists with a record of the growth record of individuals for many decades. It is based on the correlation of the distance between annuli with the growth rate of the animal; since the annual temperature cycles affect the growth rates of the fish, the age of the individual can be assessed with this method. This principle is the basis of determining growth rates from other calcified tissues of the fish body. On the assumption that the cells on the scales remain viable *in vitro,* the rate of uptake of glycine by scales, which have been removed from the animal and incubated in a medium containing radio-labelled glycine, have been shown to be highly correlated with the actual growth rate of fish under various environmental conditions (*see* Ottaway and Simkiss 1977; Adelman 1980; Goolish and Adelman 1983; Adelman 1987).

Since the major component of the increase in the mass of the animal is in the synthesis of muscle protein, there have been two dominant experimental methods to assess the rates of protein synthesis as indicators of growth in fishes. The use of RNA:DNA ratios as an indicator of protein synthesis, and thus the growth rate of the animal, is based on the assumption that while the DNA content of a cell remains constant, the RNA content varies with the degree of protein synthesis in that cell (*see* Bulow 1987). It has been determined that white muscle, over other tissues such as red muscle or liver, gives the best correlates with actual growth rates of the animal, although many environmental factors can affect this ratio. The reader is referred to the review of the various techniques used in the measurements of these nucleic acids by Buckley and Bulow (1987). The assessment of actual protein synthesis can be determined by the introduction of radio-labelled amino acids into the fish and the subsequent determination of the amount of radioactive proteins incorporated in the muscle tissue (Haschemeyer and Smith 1979).

While these assessments, through their high correlation with growth, provide a snapshot of the past or present growth record, they are not in common use in aquaculture as the methodology is often complex and the required instruments are specialized and expensive. They are more suited for research purposes rather than for the determination of growth rates in production lots of fish.

### *A Simple Growth Model for Salmonids in Aquaculture*

There have been a number of growth models published in the last three decades. While the models such as that of Stauffer (1973) and Corey et al. (1983) attempt to quantify the relationships between key rearing factors such as ration or temperature with growth, they tend to be relatively complex compared to other models developed for predictive purposes (e.g., Elliot 1975). Iwama and Tautz (1981) developed a simple growth model for salmonids in intensive aquaculture conditions. The objectives for developing that model was to provide fish culturists and biologists with a mathematical tool that was easy to use and one that could provide a high degree of accuracy in making growth projections. It has proved to be a useful tool for aquaculturists to make growth predictions with simple calculators, or in production planning models on computers. It was also easy to modify, so that it could be adjusted to any data set in which there was undisturbed growth. Subsequent predictions with the adjusted model could be made more accurately for various growth conditions (e.g., species, stock, site, feed, loading density). This section briefly presents the model, and demonstrates how the model can be used to make some basic predictions. Finally, a parameter called the growth coefficient, Gc, can be calculated from the model. It compares the actual growth rate, represented by the data, with a theoretical growth rate, calculated by the model; a Gc of unity means that the fish were growing according to the model. By utilizing Gc, comparisons among different data sets from various experimental or production conditions could be made. More detail about Gc is presented in a section below.

The model is based on two assumptions: that the cube root of weight ($Wt^{1/3}$) increases linearly over time and that the effect of temperature on growth is linear between 5 and 15°C (*see* Iwama and Tautz 1981 for justification of these assumptions). Another basic limit to this model is that it applies to periods of undisturbed growth. This latter condition limits the predictive value of this model to periods such as swim-up to smolt or from ponding to sexual maturity. Husbandry events such as sampling for inventory, marking, or disease outbreak may also disturb growth such that a new growth curve should be started once the population of fish is feeding normally.

The basic form of the model is:

$$Wt_f^{1/3} = Wt_i^{1/3} + T/1000 \times \text{Time} \qquad (2)$$

where $Wt_f^{1/3}$ is the cube root of the Final Weight in grams; $Wt_i^{1/3}$ is the cube root of the Initial Weight in grams; T is the Temperature in °C; and Time is in days.

By taking the right hand side of equation (2) to the power of 3, predictions of final weight over the specified time period can be made given the initial weight and temperature. In the course of developing this simple growth model, a linear effect of temperature on growth rate became apparent between 5 and 15°C. This is called the Growth Slope, or Gs, and was found to be approximately equal to the temperature, in °C, divided by 1000, or $Gs = T(°C)/1000$. The model can be rewritten in the following way with this substitution.

$$Wt_f^{1/3} = Wt_i^{1/3} + Gs \times \text{Time} \qquad (3)$$

### *Application of the Model*

The model allows three basic predictions; those of final weight; those of temperature and those of time, the three variables in the equation. Simple rearrangements of the basic form of the equation (2) allow those solutions. Equation (3) is the form to apply to *weight predictions*. The *average temperature required* to achieve a specified final weight from a given initial weight in a certain time interval can be calculated by equation (4), and the *time required* to achieve a certain weight, given the initial weight and average temperature can be calculated by equation (5). The calculation of average temperature should be weighted by the number of days at each temperature. One should, furthermore, use at least four decimal places if the decimal equivalent of 1/3, the weight exponent, is used (Iwama 1982).

$$T = [(Wt_f^{1/3} - Wt_i^{1/3})/\text{Time}] \times 1000 \qquad (4)$$

$$\text{Time} = (Wt_f^{1/3} - Wt_i^{1/3}) / (T/1000) \qquad (5)$$

### *The Growth Coefficient and How to Adjust the Model*

Equation (3) can be rearranged to solve for the growth slope, Gs. Since Gs was also found to approximate T(°C)/1000 (Iwama and Tautz 1981), as stated above, a calculated $Gs'$ can be derived from the associated temperature data. One can now obtain an index of how the actual Gs, calculated from actual weight and time data, compares with the theoretical $Gs'$ (T/1000) by taking a ratio. That ratio was defined as the Growth Coefficient, Gc (equation (6); Iwama and Tautz 1981). A Gc value of unity would suggest that the fish were growing according to the model. Accordingly, values above and below unity indicate higher and lower growth than what the model would predict, respectively. Regardless of the value, Gc represents a correction factor that can be applied to the original equation as shown in equation (7).

$$Gc = Gs/Gs' \qquad (6)$$

$$Wt_f^{1/3} = Wt_i^{1/3} + (T/1000 \times Gc) \times \text{Time} \qquad (7)$$

These corrected equations should make accurate predictions for any of the three variables, weight, temperature and time, for the growth conditions under which Gs was calculated. Gc may then serve as a tool for comparing different data sets, where weight, temperature, and time are known. The fact that temperature is included as a parameter in the calculation of Gc presents an advantage over the simple comparison of growth rates, where weight and time may be known, but where differences in temperature severely limit the data sets that can be compared. Tables 1 and 2 show the estimated Gc for a number of unpublished data sets. Table 1 shows the Gc values that were calculated for various growth data of salmonids reared in government hatcheries in British

**Table 1**

Growth coefficients (Gc) for various stocks of salmonids. Gc=(Gs × 1000)/ T °C (from Iwama and Tautz 1981).

| Species | Source[a] | | Year | Avg. T °C | Gc |
|---|---|---|---|---|---|
| Steelhead trout | FVTH | Qualicum stock | 1978/79 | 13.0 | 1.000 |
| | FVTH | Alouette stock | 1978/79 | 13.0 | 1.000 |
| | FVTH | Vedder stock | 1977/78 | 11.3 | 1.089 |
| | FVTH | Vedder stock | 1978/79 | 12.0 | 1.058 |
| | RCH | Robertson stock | 1975/76 | 15.9 | 0.899 |
| | RCH | Robertson stock | 1977/78 | 18.0 | 0.972 |
| | QURH | Quinsam stock | 1976/77 | 10.9 | 0.954 |
| | QLRH | Qualicum stock | 1974/75 | 12.0 | 1.008 |
| | CRH | Capilano stock | 1972/73 | 9.9 | 0.919 |
| | CRH | Capilano stock | 1973/74 | 9.3 | 0.936 |
| | CRH | Capilano stock | 1974/75 | 8.7 | 0.977 |
| Brook trout | | Haskell (1948) | | 8.3 | 1.289 |
| | | Elliot (1975) | | 15.0 | 0.533 |
| | | Elliot (1975) | | 9.5 | 0.695 |
| | | Elliot (1975) | | 5.6 | 0.304 |
| Sockeye salmon | | Brett and Shelbourn (1975) | | 15.0 | 0.840 |
| Chinook salmon | | Banks (1981) | | 15.5 | 0.955 |
| | | Banks (1981) | | 12.8 | 0.984 |
| | | Banks (1981) | | 10.0 | 0.930 |
| | | Banks (1981) | | 7.2 | 0.972 |

[a] FVTH, Fraser Valley Trout Hatchery; RCH, Robertson Creek Hatchery; QURH, Quinsam River Hatchery; QLRH, Qualicum River Hatchery; CRH, Capilano River Hatchery.

Columbia. Table 2 shows Gc values which were calculated for growth data from two Pacific salmon species reared in saltwater net cages in British Columbia. The overall averages were generally between 1 and 2, although there were significant fluctuations over the year. The growth coefficients from the more recent data set from the commercial salmon farms (Table 2) clearly show higher growth than those from the salmonids grown in fresh water (Table 1). Although the cause for this difference cannot be determined, significant factors likely include salinity, feed quality, selection, and culture conditions

Table 2

Mean Growth Coefficients (Gc) for chinook and coho salmon grown on commercial salmon farms in seawater net cages in British Columbia, Canada. Data are from 32 data sets in the period 1986 to 1989.

| Month | Chinook | Coho | Temperature °C |
|---|---|---|---|
| January | 1.783 | 2.026 | 8.0 |
| February | 1.318 | 2.216 | 8.1 |
| March | 1.529 | 1.371 | 8.3 |
| April | 1.081 | 1.166 | 8.7 |
| May | 1.616 | 1.977 | 10.4 |
| June | 1.889 | 2.115 | 11.8 |
| July | 1.753 | 1.832 | 15.4 |
| August | 1.765 | 1.996 | 15.2 |
| September | 2.055 | 1.511 | 14.3 |
| October | 2.246 | 1.432 | 12.2 |
| November | 2.292 | 2.894 | 10.0 |
| December | 1.761 | 1.655 | 8.7 |

such as rearing density. Figure 4 shows Gc values of a number of salmonid species as a function of age (or time) from a large number of published data sets. The significant scatter in the data precludes any conclusions about species or water salinity effects on this parameter. The data, however, does scatter around unity. There also appears to be a grouping above unity in fish that are over 1 year in age in both fresh water and salt water. The following section reviews the various factors that can affect growth in fishes. Where possible, Gc for various data sets from the literature are presented so comparisons among data could be made.

## FACTORS THAT AFFECT GROWTH IN SALMONIDS

This section attempts to describe the effects various factors can have on growth. Not all the factors discussed here are environmental factors. Some of the factors such as size, species and stock are intrinsic factors, whereas others are factors that are specific to husbandry conditions, such as rearing density. In attempting to discern those effects, it became evident that, taken as a whole, the current published data on growth contained such variability that rigorous comparisons were not possible. Most of the comprehensive reviews of growth in fishes, mentioned at the beginning of this chapter, have addressed the effects of the environmental factors (below) on growth. It may be useful to consider the various factors according to the categories set forth by Fry (1971), and discussed in

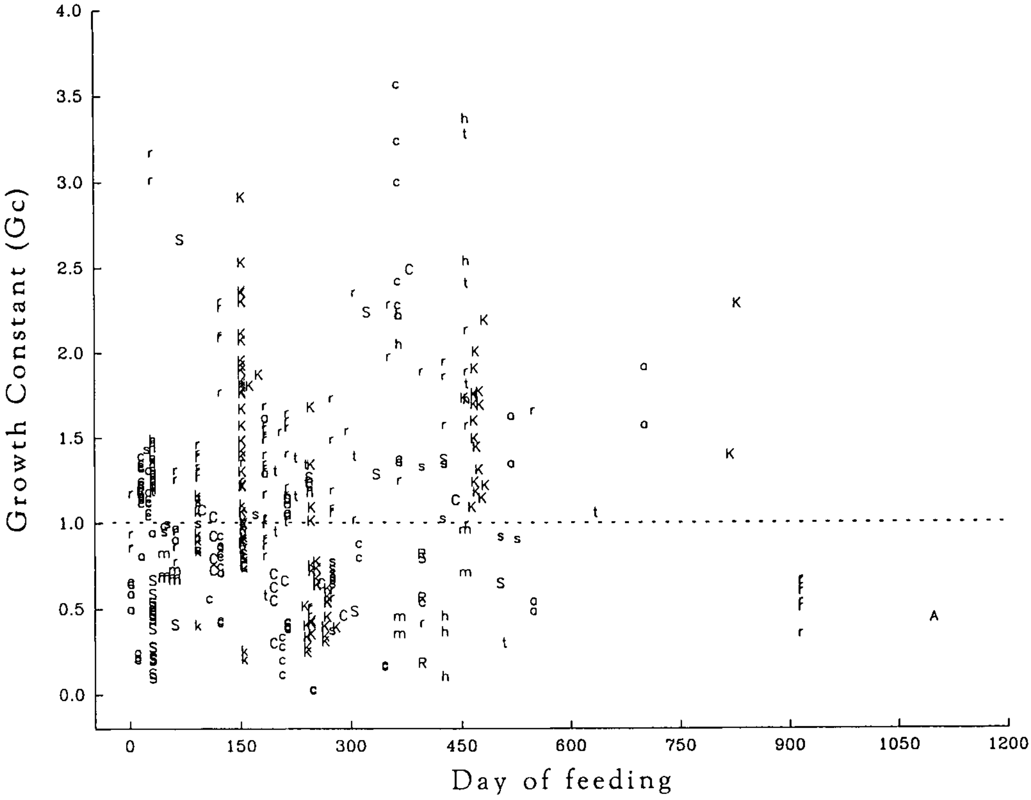

FIGURE 4. Growth constant (Gc) of various fish groups. Age of fish shown is the approximate number of days elapsed since commencement of feeding. Points are compiled from many studies (*see* references marked with an *) and show control or "normal" growing fish. The dotted line show Gc equal to 1.0. Individual points on the plot are shown as letters with A,a representing *Salmo salar*, H,h for *Salvelinus alpinus*, T,t for other trout species, R,r for *Oncorhynchus mykiss*, K,k for *O. tshawytscha*, C,c for *O. kisutch*, M,m for *O. masou*, S,s for other salmon species. Lowercase letters indicate fish groups in fresh water while uppercase letters indicate those in salt water.

detail by Brett and Groves (1979). Accordingly, controlling factors govern the rates of reactions at the cellular and molecular levels. Environmental temperature is an example of a controlling factor, and likely affects the poikilothermic fish at all levels. Although pH is another controlling factor, it is regulated at various set levels in both intracellular and extracellular compartments. Limiting factors limit the supply or removal of materials necessary for growth. Limiting factors would include those such as ration and the dissolved oxygen in the water. As the bioenergetic equation above shows, ration is the exclusive source of energy for the animal. Once the food is consumed, the process of aerobic metabolism is dependent on an adequate supply of oxygen at the cellular level. Masking factors modify or prevent the effects of the above factors by some regulatory mechanism. Water salinity, as a masking factor, may reduce the effect of ration on growth by increasing the energetic demands on the animal, through ionic and osmotic regulatory processes needed to maintain homeostasis in the face of a change in water salinity. Directive factors such as changes in daylength can elicit dramatically different responses of the fish to environmental factors. For example, an increase in daylength stimulates growth and has been recognized as a key environmental cue in the initiation of smoltification. These categories may present some structure in which to view the factors below that can be considered as environmental factors.

### *Temperature*

Of all environmental factors that influence growth of fishes, water temperature exerts the single most dominant effect. Inasmuch as chemical reactions drive every aspect of the bioenergetic model discussed above, temperature has a profound effect on the growth of fish. As a controlling factor (Fry 1971), environmental temperature affects the rate at which those chemical reactions take place in the poikilotherm. Where the effects of a number of environmental factors such as ration and photoperiod have been studied simultaneously with temperature, temperature not only showed the largest relative effect on growth, but it has been found to influence the rate at which the other factors affect growth. An example of this is the multifactorial experiment of Clarke et al. (1981) in which they studied the effects of temperature, photoperiod, and salinity on growth and smoltification in coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*), and sockeye (*O. nerka*) salmon.

Each species has a range of temperatures which it can tolerate (*see* Chapter 2), limited by the lethal boundaries of metabolic depression and torpor at the low end and metabolic dysfunction at the high end. As the temperature increases from minimum, growth rate increases to a peak, and declines with further increases up to a maximum. An example of this trend is shown in Figure 5 for sockeye salmon from the model study of Brett et al. (1969). The increasing temperature stimulates appetite as well as the processing of the consumed food. Up to the optimum temperature, where the growth rate is maximum, the net effect of temperature is for anabolic processes to exceed catabolic ones. After that point, the temperature-related increase in metabolic rate may exceed the ability of the system to supply the essential substrates and oxygen to the respiring tissues. Temperatures beyond the optimum range for the fish, both below and above, would also result in suboptimal function of essential enzymes as well as disturb acid-base balance. It is not surprising that no clear trends are evident in the relationship of Gc values as a function of temperature within the optimum range for salmonids (Figure 6), as the data set is a conglomerate of various salmonid species in both freshwater and saltwater environments.

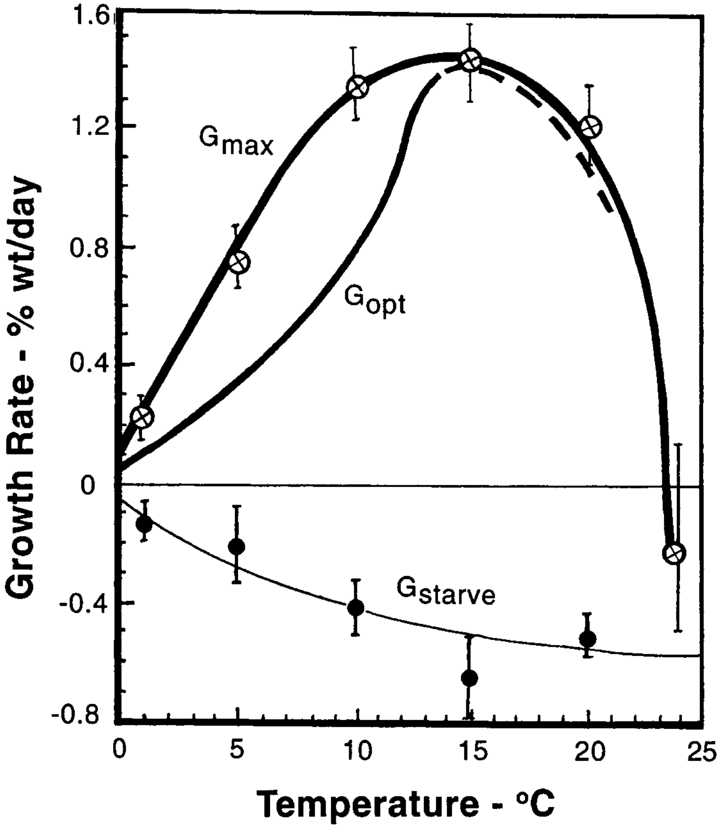

Figure 5. Relations of various defined growth rates according to maximum ration ($G_{max}$, $R_{max}$), optimum ration ($G_{opt}$, $R_{opt}$), maintenance ration ($G_0$, $R_{maint}$), and starving ($G_{starv}$, $R_0$). Means ± 2SE (from Brett et al. 1969).

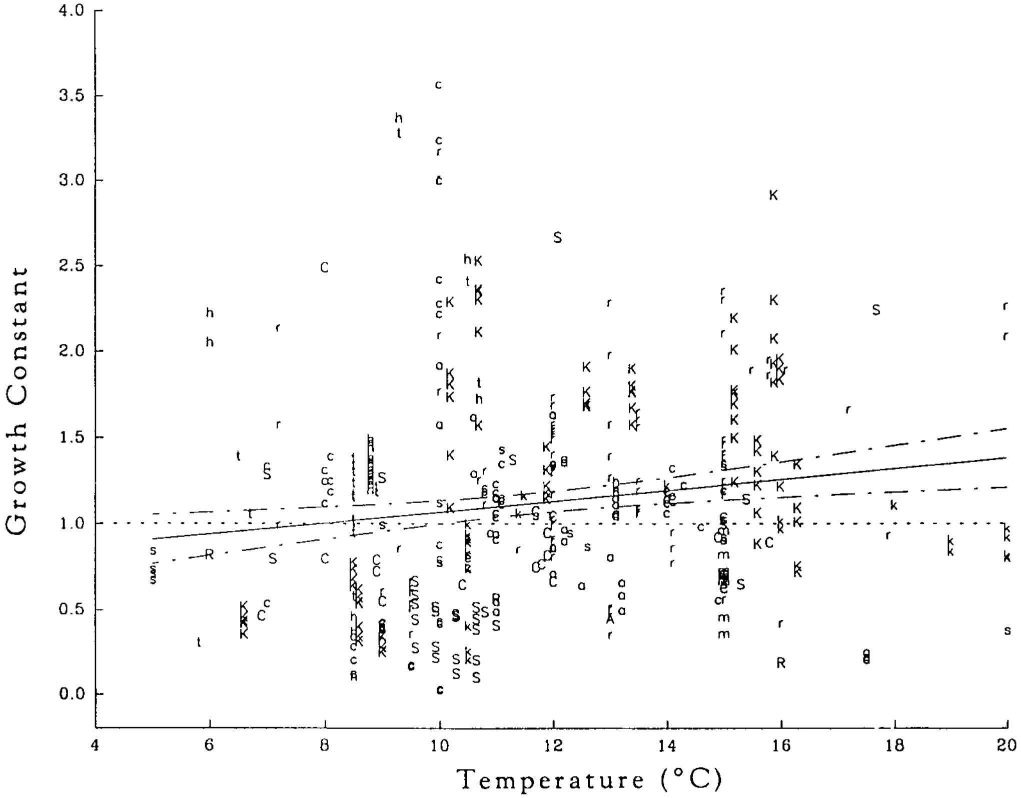

Figure 6. Growth Constant (Gc) of fish groups reared a temperatures ranging from 5 to 20°C. Points are compiled from many studies (*see* references marked with an *) and show control or "normal" growing fish. Dotted line indicates Gc=1 while solid line shows the regression line (slope 0.031 and r=0.15) with its 95% confidence limits as dot-dash lines. Individual points on the plot are shown as letters with A,a representing *Salmo salar*, H,h for *Salvelinus alpinus*, T,t for other trout species, R,r for *Oncorhynchus mykiss*, K,k for *O. tshawytscha*, C,c for *O. kisutch*, M,m for *O. masou*, S,s for other salmon species. Lowercase letter indicate fish groups in fresh water while uppercase letters indicate those in salt water.

The data generally clustered around the Gc value of 1.0. It is perhaps interesting that there was a positive slope in the best-fit line through the data set and that it crossed the line of unity at about 8°C. Although this means that the growth performance, relative to the theoretical growth predicted by the model, changes with temperature, the limitations of such a simple predictive growth model precludes meaningful speculation about the nature or cause of such a changing relationship with temperature. The optimum temperatures for the various salmonid species are presented in Table 3 (*see also* Chapter 2).

Fluctuating temperatures have been shown to have a stimulatory effect on growth. Berg et al. (1990) found that growth of Atlantic salmon (*Salmo salar*) was significantly higher under a rhythmically alternating temperature regime, compared to controls which were grown at a temperature close to the mean of that temperature range. In contrast, however, Clarke et al. (1978) found that fluctuating temperature had no effect on the increase in weight or length of coho or sockeye salmon. It may be that the absolute values of the fluctuating temperatures, in relation to the optimum temperature for growth, are important factors in this regard. When the mean of the fluctuating temperature regime was maintained lower than the constant control temperature set at the optimum for growth, growth was significantly improved in rainbow trout (*Oncorhynchus mykiss*) (Hokanson et al. 1977), underyearling sockeye salmon (Clarke et al. 1978), and juvenile striped bass (*Morone saxatilis*) (Cox and Coutant 1981; cited by Brett and Groves 1979). In an attempt to explain the higher growth rates of brown trout (*Salmo trutta*) in Norwegian rivers, compared to that predicted by Elliot's (1975) growth model, Jensen (1990) speculated that the fluctuating temperatures of the natural environment might have been a significant component. As growth was better in the spring than in the fall, he considered that the direction of overall change in the fluctuating temperature regime was significant; that is, the regime of increasing temperatures stimulated growth more than the declining trend in the fall. As Spigarelli et al. (1982) found that food intake was two- to three-fold higher under conditions of fluctuating temperatures compared to constant temperature, it is probable that this might be the mechanism whereby the above phenomenon occurred.

### *Ration and Feeding*

The food that is consumed represents the sole source of energy to the fish. It is therefore a vital component of the energy budget, as discussed above. The data discussed below focuses on the effects of ration and feeding strategy on growth in salmonids. Figure 7 shows the Gc values for rainbow trout, chinook salmon, and sockeye salmon as a function of ration levels. It is clear that growth rates have a strong positive correlation with ration levels. Furthermore, evidence exists for factors other than temperature and ration (e.g., species and water salinity-related factors) that affect the growth response of the animals to ration. Other than the rainbow trout fed at 2% body wt/day in fresh water and the groups of chinook salmon grown in salt water, many of the data sets show Gc values near unity. The benchmark studies of Brett in the 1970s on the relationships among ration, growth, temperature, and other environmental and physiological factors in sockeye salmon served to demonstrate the complex nature of those relationships, and at the same time provided a model approach to investigating such topics (*see* Brett and Groves 1979).

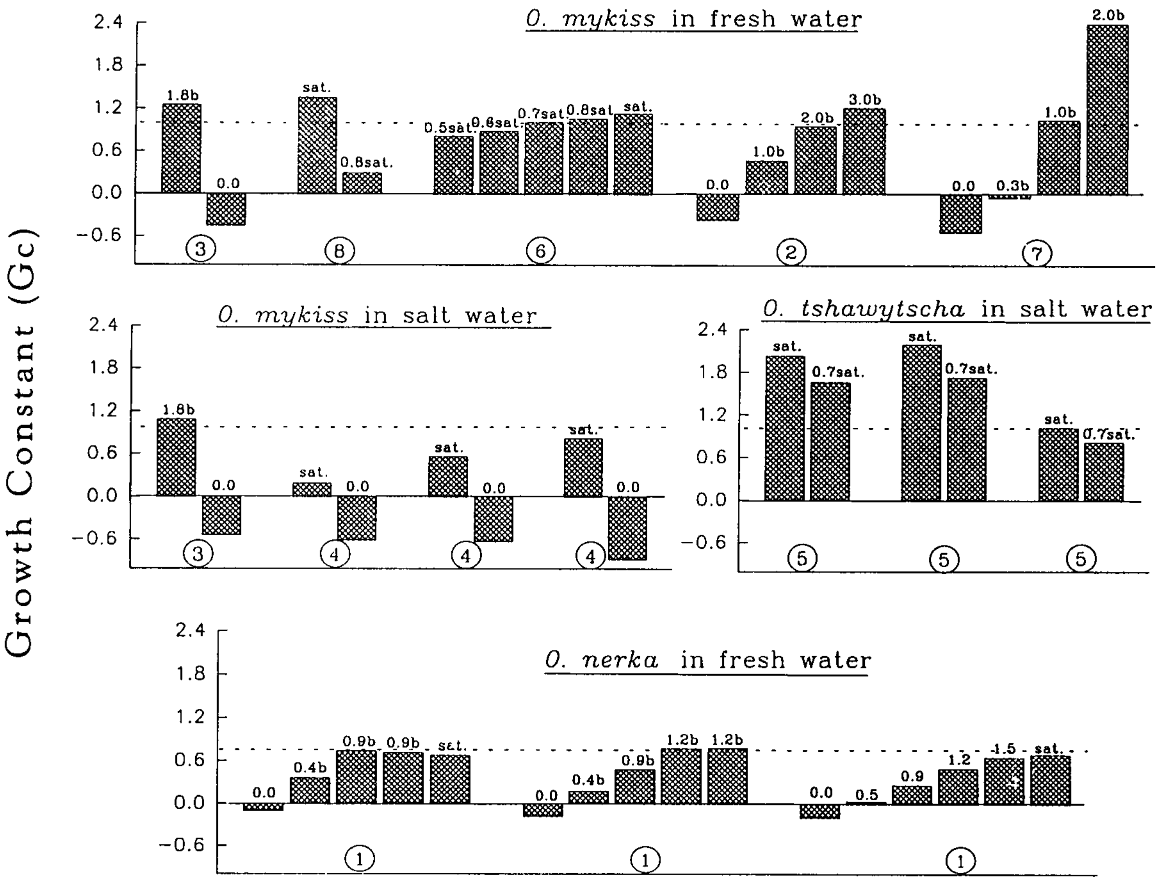

Figure 7. A series of graphs adapted from a number of experimental studies of the effect of feeding rate on growth. Plots arranged by species and water salinity with clusters of bars showing growth coefficient (Gc) within a study. The feeding rate associated with each group of fish is shown on the top of bars as percent dry body weight (b) or satiation (sat.) that was fed. References are given as a number (within circles). Reference numbers are as follows: 1 Brett et. al. 1969; 2 Huisman 1976; 3 Jürss et al. 1986; 4 Jürss et al. 1987, 5 Mazur 1991; 6 Smith 1987; 7 Storebaaken et al. 1991; 8 Weatherly and Gill 1981.

**Table 3**

Optimum temperature for growth in various salmonid species.

| Species | Optimum | Salinity | Initial Size | Reference |
|---|---|---|---|---|
| Brown trout[a] | 12.8 | Fresh | 300 g | Elliot (1975*c*) |
| Chum salmon[a] | 13.0 | 35‰ | underyearling | Kepshire (1971) |
| Brook trout[a] | 14.0 | Fresh | 0.1-0.2 g | McCormick et al. (1972) |
| Arctic grayling[a] | 14.0 | Fresh | yearling | Swift (1963) |
| Sockeye salmon[a] | 15.0 | Fresh | 6-20 g | Brett et al. (1969) |
| Chinook salmon[a] | 15.5 | Fresh | 2-9 g | Banks et al. (1971) |
| Pink salmon[a] | 15.5 | 35‰ | underyearling | Kepshire (1971) |
| Rainbow trout[a] | 17.2 | Fresh | 0.3-3.0 g | Hokanson et al. (1977) |
| Huchen | 17.0 | Fresh | underyearling | Jungwirth et al. (1989) |
| Atlantic salmon | 14.5 | Fresh | | Dwyer and Piper (1987) |

[a] Extracted from Table I of Brett and Groves (1979)

The information that came from J.R. Brett and his collaborators have contributed not only to our basic understanding of fish physiology, but to the development of intensive salmonid aquaculture technology. While it is clear that the quality or the content of the food can affect performance, optimal or acceptable diet formulations for most salmonids have been developed and the feeding schedules which are available from feed manufacturers should provide for adequate growth. In examining the effects of dietary protein content and feeding rate on performance in rainbow trout, Reinitz (1987) recommended that adjusting feeding rates was more economical than making adjustments to the crude protein and crude fat levels in the feed. Those recommended feeding levels should, however, be adjusted according to local conditions, as higher feeding frequencies have been shown to be necessary under conditions of high density (Holm et al. 1990) and physical activity (Christiansen and Jobling 1990).

Consistent with the phenomenon of compensatory growth in mammals and birds (*see* Wilson and Osbourne 1960; Thornton et al. 1979), there is evidence that intermittent feeding, or switching between restricted and satiation feeding, can result in physiological adjustments to the digestive system such that certain efficiencies are achieved in terms of growth (Bilton and Robins 1973; Smith 1981; Weatherley and Gill 1981; Dobson and Holmes 1984; Kindschi 1988; Miglavs and Jobling 1989; Quinton and Blake 1990). The end result is the attainment of overall growth comparable to fish on a constant feeding regime. Feed conversion efficiency is, therefore, increased because only a fraction of the ration fed to fish on constant feeding is used. Quinton and Blake (1990) demonstrated this trend in rainbow trout fed intermittently, on a three-week-on and three-week-off cycle. The fish lost weight during the starvation period, but made up the lost weight and caught up with the control fish through extraordinarily high growth rates when feeding was resumed. Although the mechanism by which the extremely high growth rates are achieved are unknown, Quinton and Blake (1990) suggested that a reduction in catabolic activity, with normal rates of protein synthesis, could explain the phenomenon; there was no a priori reason to assume some novel mechanism to explain the enhanced rates of protein synthesis.

While the results of some studies on compensatory growth are promising, not all studies have given consistent results, although the differences could be due to the different treatments and experimental conditions. In contrast to most studies on compensatory growth in fishes, Kindschi (1988), working with intermittent feeding in rainbow trout, showed that fish on intermittent feeding grew to significantly smaller sizes than control fish on continuous feed. Miglavs and Jobling (1989) showed that there was a growth spurt in Arctic charr (*Salvelinus alpinus*) when they were switched from a restricted ration to satiation feeding. Quinton and Blake (1990) found that the schedule of starvation and feeding had a significant effect on the effectiveness of this technique to increase conversion efficiency. They found that fish on a three-week-on and three-week-off schedule grew to a size equal to fish on continuous feed, saving feed by a factor of 2.35 to 2.64. Durations of either starvation or feeding less than three weeks did not yield this benefit. Data from Kindschi's (1988) experiment supports this in that the fish held on a four-week-off and four-week-on feed regime grew to the largest size among the experimental groups; although the absolute mean size was only about half that of the control fish. Fish on a schedule of four-days-off and three-days-on feed grew to about one third of the control fish by the end of the experiment. Quinton and Blake (1990) also found that the fish on the experimental feeding schedule did not differ significantly in carcass composition, compared with control fish. Storebakken et al. (1991) also found feeding levels to have little effect on the proximate composition of rainbow trout. Growth rates after starved fish catch up to fish that have been on continuous feed have been shown to be similar to control fish in sockeye salmon (Bilton and Robins 1973) and rainbow trout (Weatherley and Gill 1981). In studying the weight changes of the whole body, as well as a number of internal organs and tissues of rainbow trout, Weatherley and Gill (1981) found that the effects of restricted ration (3% of dry body weight/d) were more similar to the effects of prolonged starvation (13 wk), than to starvation of a shorter period (3 wk). This latter finding is an important consideration if ration restriction, or starvation is being considered as a management technique to control growth rates in fish.

As discussed in the section below on the relationship between growth rate and sexual maturation, the manipulation of growth rates through ration to suppress precocious sexual maturation might be an attractive technique to fish culturists. Furthermore, the manipulation of feeding to reduce the final size of hatchery-reared fish, so that they approximate more closely the wild fish, might be considered by fish culturists and managers involved in the application of intensive fish culture for the enhancement of wild stocks. While these are possible applications of knowledge about restricting ration or about starvation, the optimum protocol should be developed that takes into consideration the species, local water conditions, and season. Even within a species, faster growing strains of rainbow trout have been shown to have lower metabolic costs than slower growing strains (*see* section below). Differences in basal metabolic rates, which may differ among species and stocks, may have significant consequences in defining the optimum ration levels for such strains. The temperature for maximum growth has been shown to decrease with rations lower than optimum (*see* Brett and Groves 1979) and fish have been shown to prefer lower temperatures when starved (Cossins and Bowler 1987). Although concentrations of cortisol, a hormone associated with stress, have been shown not to increase in response to starvations of one month or six weeks in rainbow trout (Sumpter et al. 1991) or three weeks in chinook salmon (Barton et al. 1988), there is little information available on the effects such treatments may have on important physiological functions such as resistance to other stressors or to disease.

### *Feed Quality*

Although it is not generally feasible or practical for fish culturists to manipulate the quality of feed, investigations into the effects of feed quality on production performance in aquaculture represents an active area of research. In the past, research concentrated on determining the critical levels of key ingredients for optimum performance. Now that those parameters are rather well defined for salmonid diets, research tends to concentrate on alternative sources of those main ingredients. For example, sources other than fish protein may be more economical and contribute to the profitability of fish farming, as feed constitutes the largest operational cost to intensive salmonid culture (*see* Chapter 10).

Most studies that have addressed this issue have shown that increasing the protein content of the feed results in increased growth. It is generally accepted that optimum growth in salmonids, during the grow-out or fingerling to adult phase, can be achieved with a protein content of 40-45% of the diet. Since this is the most expensive component of the feed, research has been conducted on adjusting other high energy ingredients, such as lipids, to spare the protein requirements of the diet. The optimum lipid level for salmonid culture is about 12-14% of the diet. It has been demonstrated that higher lipid levels of 15-20% resulted in higher protein utilization in trout and carp (*Cyprinus carpio*) (Davies 1989; Lee and Putnam 1973; Takeuchi et al. 1978; Watanabe et al. 1979; *see* Steffens 1981). This efficiency resulted from a higher Protein Efficiency Ratio (PER) and Productive Protein Value (PPV). Ogata and Murai (1991) showed that in masu salmon (*Oncorhynchus masou*), growth was proportional to the level of lipid in the diet, provided the protein content of the diet was adequate (42%). Lipid had no effect at a protein level of 24%. It has been suggested that the appetite of fish is regulated by the energy content of the food (Cho et al. 1976; Grove et al. 1978), so the energy content represented by protein could be replaced by lipids (Medland and Beamish 1985; Beamish and Medland 1986) as long as the protein content is above 30% (Gropp et al. 1982). Dosanjh et al. (1988) showed that supplementing the diet with various lipid sources, such as pork lard, herring oil, and canola oil, did not affect the growth of juvenile fall chinook salmon in fresh water. Forster et al. (1988), however, demonstrated that growth in juvenile coho salmon was depressed with the inclusion of oxidized herring oil in the diet. They speculated that the cause may have been the reduced digestibility of the oxidized oil and suboptimal concentrations of ω3 fatty acids. A 220% increase in weight gain, and a 160% improvement in conversion efficiency were observed in underyearling masu salmon by Ogata and Murai (1989) in response to the replacement of the fatty acids, palmitate and oleate, with linoleate and linolenate, although replacement with linoleate alone had no effect on growth.

Supplementation of the diet with various ingredients has had a variety of effects on growth, depending on the added material. Supplementing the diet with vitamin E, at 30 and 1030 IU/ kg, did not affect the growth of juvenile coho salmon (Forster et al. 1988). McBride et al. (1982) reviewed the literature pertinent to the effects on growth in salmonids of adding various hormones in the feed. With the exception of 4-cholorotestosterone, the additions of androgens at 1-10 mg/kg of feed have resulted in growth enhancement in sockeye, coho, and chinook salmon. These treatments, however, have not been effective with rainbow trout and Atlantic salmon. Estrogens are generally ineffective growth promoters (*see* McBride et al. 1982). McBride and Fagerlund (1973) showed that the effectiveness of growth enhancement for the *Oncorhynchus* species was greatest with 17-$\alpha$ methyltestosterone (MT), followed by 11-ketotestosterone, and then by testosterone. Fagerlund et al. (1983) demonstrated that the addition of MT to the diet enhanced growth in coho salmon, regardless of the diet tested. MT increased the appetite of the fish in July and August. In contrast, Dickhoff et al. (1991) found no stimulation of growth in this species with the administration of MT. Appetite may be enhanced by anabolic steroids (*see* McBride et al. 1982). The supplementation of diets with 3-5-3 triiodothyronine ($T_3$) has been shown to enhance growth rate in Atlantic salmon, in a dose dependent manner (range, 0 to 100 ppm), by Saunders et al. (1985); physical abnormalities, however, resulted at the highest dose. Stimulation of growth by $T_3$ has also been effective in coho, chinook, and Atlantic salmon (*see* McBride et al. 1982). The addition of 10% krill to the feed of broodstock to increase the pigmentation level of the offspring had no effect on growth of those fry. The addition of propylene glycol as a corn starch supplement in low-energy diets had no effect on growth in rainbow trout (Hilton et al. 1986). In fry of Atlantic salmon, Poston (1990) found that the addition of lecithin from a number of sources (pure soy lecithin at 4% of the diet; feed-grade lecithin at 8% of the diet), all enhanced growth by about 50% if fed to the fish before they reached 1.7 g. The fry grew better, deposited more fat and showed higher conversion efficiencies.

The requirements of a few minor ingredients have been assessed in a number of studies. Poston (1986) showed that growth in rainbow trout was best in diets with L-methionine, followed by diets with DL-methionine. Analogs of methionine also enhanced growth, but not to the same degree as these natural forms. Methionine is one of the most limiting amino acids in fish diets, especially when plant proteins are used.

In an assessment of tryptophan requirements, Kim et al. (1987) showed increased growth, nitrogen retention, and feed conversion efficiency in rainbow trout with increased tryptophan content from 0.06% to 0.20% of the diet. It has been shown in chinook salmon that potassium ($K^+$) at a concentration of 0.8% of the diet, is required for maximum growth in that species. A range of 0.6 to 1.2% [$K^+$] in the diet provided sufficient $K^+$ to maintain adequate whole-body $K^+$ levels, taking into account the availability of $K^+$ from the water (*see* also Sakamoto and Yone 1978). Studies of the requirements for ions in the diets are complicated by the fact that fish have significant abilities to transfer ions between body fluids and the environment. Given this, it is not surprising that Wilson et al. (1985) found that dietary electrolyte imbalances did not affect growth or feed conversion efficiency in juvenile rainbow trout.

### *Salinity*

The relationship between water salinity and growth in salmonids can be considered in the context of the energetic cost of ionic and osmotic regulation. The maintenance of blood ion concentrations, in the face of large ionic gradients between blood and water, requires the active uptake or excretion of ions to compensate the passive diffusion of those ions down their electrochemical gradients, through the permeable epithelia (e.g., gills and intestinal tract) of the body. The blood concentrations of sodium ([$Na^+$]) and chloride ([$Cl^-$]) in resting salmonids are approximately 145-155 mEq/l and 100-110 mEq/l, respectively. Therefore, in fresh water, the chemical gradients for $Na^+$ and $Cl^-$ from blood to water may be 100 to 1, whereas those gradients from seawater to blood would be approximately 3 to 1; the exact values being dependent on the actual ionic concentrations in the blood and water. There may be associated costs for osmoregulation, since ionic and osmotic regulation are intimately related. In fresh water, the concentration differences between blood and water create an inward osmotic gradient. The influx of water is balanced with the production and excretion of a large volume of dilute urine. Conversely, in seawater, the osmotic loss of body fluids is compensated by the fish drinking seawater. This exacerbates the problem of gaining ions from the water, in addition to those entering through the permeable epithelium of the gill. The data from the literature does not show a consistent pattern as to the effect of salinity on growth. Although there might be anecdotal evidence to suggest that growth in salt water is consistently higher than that in fresh water, as Figures 3, 4, and 7 show, the highest growth rates and Gc values, respectively, are not always in fish in salt water. The Gc values as a function of salinity (fresh or salt water), by species in Figure 7, as well as Gc values in other figures show that other factors, also exert significant influences on salmonid growth.

The experiments specifically designed to describe the relationship between water salinity and growth have also provided a range of responses. While there is a general trend for growth to decline with increases in water salinity, this response varies with species, age, and season. Lower growth rates were observed in coho salmon pre-smolts

(Otto 1971) and Atlantic salmon parr (Saunders and Henderson 1969*a*) in salinities of 20‰ compared to fresh water. Very high salinities also have been shown to depress growth rates in juvenile chinook salmon (33‰ Kepshire and McNeil 1972), rainbow trout (30‰ Ericksen 1978; cited by McKay and Gjerde 1985), and Atlantic salmon smolts (30‰ Saunders and Henderson 1969*a*). McKay and Gjerde (1985) attributed the difference in their data from that of Eriksen (1978) to seasonal effects in rainbow trout. While McKay and Gjerde (1985) found growth rates to decline with increasing salinity from fresh water to 32‰, during the months from February to May and September to December, Eriksen (1978) observed growth rates to be higher in 10‰ salinity than in fresh water in the months from July to October. Season may affect the appetite, thus food intake, of the fish. High growth rates around salinities near isotonic for fish blood have been reported in several studies. Bullivant (1961) found growth rate of yearling chinook salmon to be higher in water of 17‰, compared to fresh water and seawater. Saunders and Henderson (1969*b*) also observed maximum growth rates in Atlantic salmon to be in brackish water in the spring but in fresh water in the fall. Under very similar conditions (starting weight 0.5 g and 10°C; static water in aquaria), Canagaratnam (1959) and Otto (1971) also found growth rates to be higher in brackish water compared with fresh water in coho salmon fry. Furthermore, weight loss was observed to be lower in rainbow trout in 8‰ salinity compared with fresh water or 12‰, when food was withdrawn (Jürss et al. 1986).

In the bioenergetic model above, the cost of ionic and osmotic regulation would be the appropriate parameter associated with the effects of salinity on growth, although the actual cost of such regulation is not clear (Morgan and Iwama 1991). Since the energetic costs associated with ionic and osmotic regulation are associated with the ionic gradients between blood and water, one could put forth the hypothesis that those costs would be minimal at isotonic salinities, and furthermore, those costs might be assessed by determining the difference in metabolic rate between isotonic and either fresh water or seawater. If the magnitude of the salinity effect was large enough, other components of the energy budget of the animal, such as growth, might be affected. Investigations have tested this hypothesis, but have yielded a wide range in the energetic cost of ion regulation. On a theoretical basis, which considered the work that would be associated with actively compensating the passive leak of ions down their ionic gradients, Eddy (1982) estimated the energetic cost of osmoregulation in salmonids to be less than 1% of resting metabolism. Shaw et al. (1975) showed that salinities from fresh water up to 20‰, had no significant effect on growth. In contrast, in experiments with rainbow trout, Rao (1968) showed a difference in metabolic rate of 20-28% between isotonic medium and both fresh water and seawater. In contrast to this model response, where the energetic minimum occurs at isotonic salinity, several studies have shown the salinity where the lowest level of metabolic rate and highest growth rate to occur was in fresh water (Shaw et al. 1975; Clarke et al. 1981; McKay and Gjerde 1985; McCormick et al. 1989; Morgan and Iwama 1991). A review of recent literature revealed that this model represents only one of several responses that have been documented (Morgan and Iwama 1991). The responses include: (1) type I - metabolic rate does not change over a wide range of salinities; (2) type II - metabolic rate is minimum in isotonic salinity and increases at lower and higher salinities; (3) type III - metabolic rate is minimum in fresh water and increases with salinity; (4) type IV - metabolic rate is highest in fresh water and decreases to the isotonic point (salinities higher than isotonic are not tolerated; and (5) type V - metabolic rate is minimum in sea water and increases with lower salinities. Euryhaline fish, which can move freely through waters of varying salinity generally show a type I response. The type II response seems to be found in euryhaline fishes that are found in fresh water as adults or in estuaries as juveniles. Fish that are found as juveniles in fresh water or in estuaries as adults generally show a type III response. Stenohaline freshwater fish seem to exhibit a type IV response, and fish that are found primarily in marine environments show a type V response. As noted by Nordlie (1978) and Brett and Groves (1979), juveniles seem more prone to salinity effects, than adults of the same species. It is also apparent that in most cases, the metabolic rate is minimum at the salinity in which the animal is normally found at that life stage.

While the growth data from individual studies might show one or more of the general patterns described above, when data from the literature on salmonids are considered together, there is no clear or consistent pattern to the effects of water salinity on the growth of salmonids (Figures 1,2,3). The species, life stage, and season all seem to be significant factors in this relationship. It is possible, furthermore, that the effects of water salinity might be expressed through metabolic effects other than, or in addition to, those associated directly with ionic and osmotic regulation, which have not yet been elucidated (Febry and Lutz 1987; Morgan and Iwama 1991). Although the mechanisms are not yet known, it is apparent that water salinity does affect the metabolic rate of salmonids sufficiently in some cases such that other components of the energy budget of the animal, such as growth, are affected. On one hand, the abrupt transfer of anadromous salmon from fresh water to seawater prior to smoltification can result in stress that can lead to death. On the other hand, the energetic gains that might be realized through the manipulation of water salinity could shunt energy to the other components of the energy budget, such as growth and reproduction. There are large gaps in our basic understanding of how salinity affects growth and metabolism in fishes, and the opportunities to apply that knowledge to improve aquaculture practice are clearly evident.

### *Species and Stocks: Genetic Factors*

There can be no doubt that there are genetic bases for growth in fishes. Investigations that address this subject fall into several categories. Some studies have described growth differences among different species of salmonids. Others have described growth differences among various stocks (used as being equivalent to strains) of salmonids. These studies have either described natural populations in their environments, or cultured populations in a common environment. A third group of studies have concentrated on demonstrating the genetic component by assessing the heritability of the additive effects through breeding experiments.

As Figure 8 shows, there are differences in growth among the various species of salmonids. While this may be interesting from an academic perspective, the species that are cultured for commercial production are dictated by the demands of the market place, and related intimately with the commercial fisheries catch. There is evidence that these species differences may be larger in magnitude than stock differences. Sadler et al. (1986) examined growth of two strains in each of rainbow, lake (*Salvelinus namaycush*) and brook (*S. fontinalis*) trout and splake (lake x brook trout hybrid). The best growth at 10°C was in rainbow trout, followed by lake trout, splake, and brook trout. At 16°C, the best growth was in brook trout, followed by rainbow trout, splake, and lake trout. It is interesting to note that the hybrid splake consistently exhibited intermediate growth to the two parent species, while stock effects were significant in lake trout and rainbow trout, but not in brook trout. Figure 9 shows the growth performance among various stocks of several salmonid species.

Several studies have examined the differences in growth, along with other parameters, among various salmonid stocks in their natural habitats. Differences in alevin and fry size of pink salmon (*Oncorhynchus gorbuscha*) from five odd-year stocks in British Columbia (BC), Canada were attributed to family effects; there were no significant stock effects (Beacham and Murray 1986). Beacham and Murray (1987) inferred different growth rates in maturing chum salmon (*O. keta*), from their final body sizes among 78 stocks in southwestern BC. Generally, large adults produced large eggs, which resulted in large fry. Four different populations of Arctic charr (*Salvelinus alpinus*) in an Icelandic lake were shown to have distinct differences in growth rate, as well as other life cycle characteristics (Jonsson et al. 1988). Differences in growth among different stocks of Arctic charr also were shown by Barbour and Einarsson (1987). Furthermore, Heggberget et al. (1986) showed that three stocks of Atlantic salmon from different sections of the River Alta in Norway showed different growth rates. While the results of these studies are difficult to interpret in attempting to discern stock effects, several studies show that stock differences persist when the environment is controlled.

In a study that involved half-sib families from six stocks of chinook salmon, the growth rates as well as body weights of alevins and smolts varied among different stocks and among families within stocks (Withler et al. 1987). Gunnes and Gjedrem (1978) showed growth differences during a two-year period of culture at sea, among 37 stocks of Atlantic salmon, grown at five different salmon farms. Each stock was represented by several full-sib and half-sib families. Heritabilities ($h^2$) of 0.28 and 0.31 were estimated for body weight and length, respectively. Since the farm component had only a slight effect on growth, they concluded that it could be ignored for the consideration of heritability estimates for purposes of breeding. In collaboration with private salmon farms and government agencies in British Columbia, Kreiberg (1987) found differences in growth among six stocks of chinook salmon grown at five different salmon farms. McGeer (1989) showed that there were differences in growth among six stocks of coho salmon reared in a single common environment. Although Ringo (1987) showed that there were no significant differences between landlocked (Takvatan) and anadromous

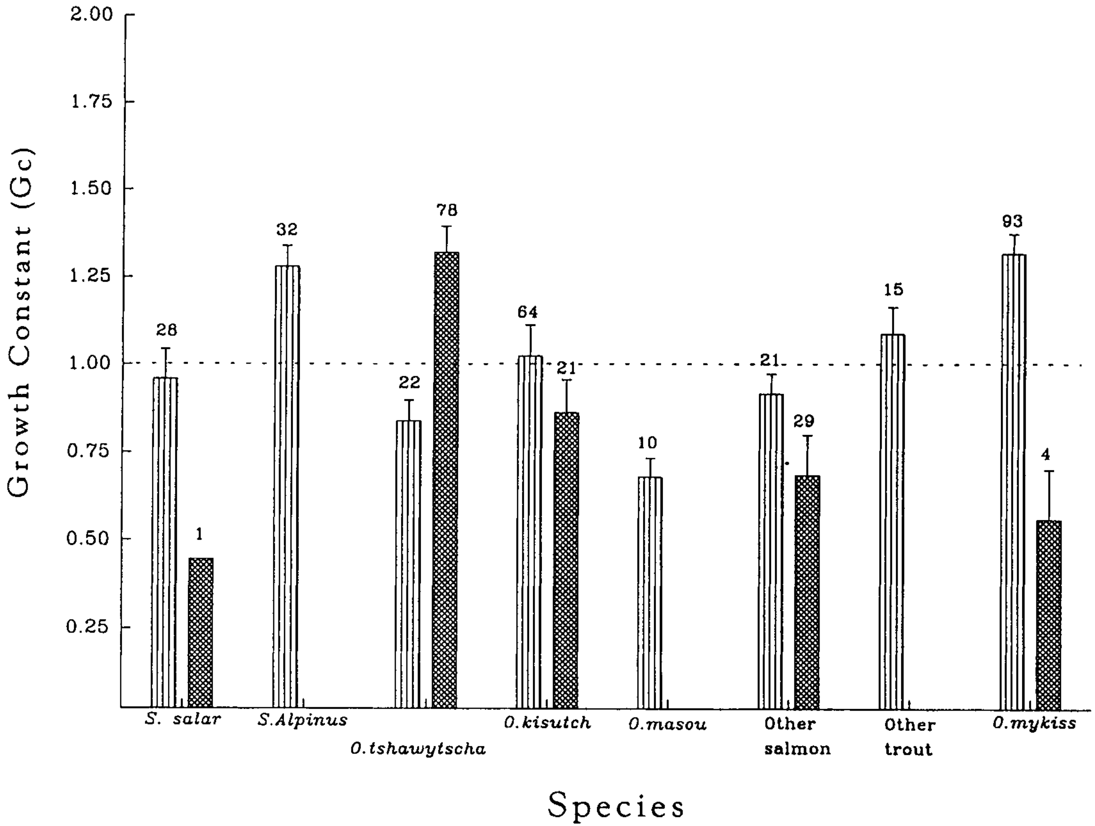

FIGURE 8. Mean Growth Constant (Gc) (+1 SE) of a variety of fish species. Species are shown by labels with vertically lined bars for fresh water means and crosshatched bars for salt water means. Means are compiled from many studies and show control or "normal" growing fish. The dotted line shows a Gc of 1.0. The number given on the top of each bar shows the number of data sets from which the mean Gc was calculated.

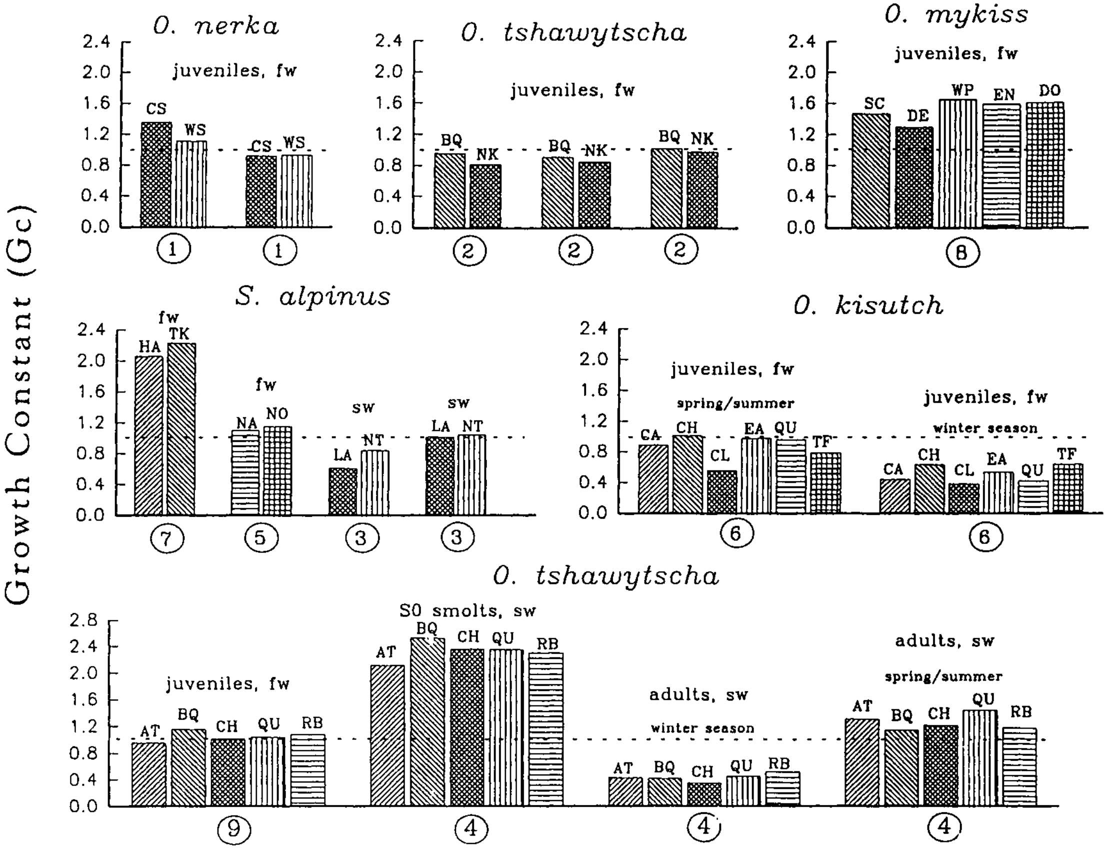

Figure 9. A series of graphs adapted from studies growth among stocks. Plots are arranged by species with brief descriptions (fw for fresh water, sw for salt water). Clusters of bars show mean growth constant of individual stocks of one study with the stock initials at the top of the bar. The dotted line shows a Gc of 1.0. The references are given as number within circles below the bars. References numbers are as follows: 1 Brett 1974; 2 Brett et al. 1982; 3 Delabbio et al. 1990*a*; 4 Kreiberg 1987; 5 de March 1991; 6 McGeer 1989; 7 Ringo 1987; 8 Smith et al. 1988; 9 Withler et al. 1987.

(Hammerfest) stocks of Arctic charr, significant differences were observed between the Labrador and Northwest Territories stocks of Arctic charr by Delabbio et al. (1990*a*). Significant stock differences in growth, as well as other parameters such as dressed weight, were also observed among ten stocks of rainbow trout (Smith et al. 1988). While the mechanisms causing these differences are not known, Kolok (1989) used fast-growing and slow-growing stocks of rainbow trout to test the hypothesis that differences in basal metabolism cause those effects. He found that the fast-growing stock had a maintenance ration that was 32% lower than the slow-growing stock. Kolok (1989) suggested that the fast-growing stock was therefore able to allocate more energy to growth (*see also* Degani and Lee-Gallagher 1985; Danzmann et al. 1987).

Breeding animals to improve growth performance is the natural application of the fact that there is a genetic basis for growth in fishes. The reader is referred to Chapter 11 in this book, as well as several review papers, mostly published in the mid-1980s, on the topic of genetics in aquaculture (*see* reviews by Gjedrem 1975, 1983, 1985; Wilkins 1981; Doyle 1983; Gall 1983; Gjerde 1986). There is general agreement that the potential for improving parameters such as growth rates and age at sexual maturation are good, due to two main reasons: (1) there is a large genetic variability in these traits in salmonids; and (2) the high fecundity of salmonids enables strong selection pressures to be exerted for these traits. Combining selection at the individual level with that at the family level is also generally recommended. Given the differences in growth performance among stocks of a given species (*see* above), the best individuals, families, and stocks should be used for selective breeding programs. In real trials, the high potential for genetically based additive effects seem to be realized in most cases. In Gjerde's (1986) review of 16 experiments on possible additive effects on production parameters through breeding, 15 experiments yielded positive responses to selection. He also recommended that estimates of genetic parameters and selection be based on growth data in fully grown fish, close to marketing. Low heritability ($\approx$0.1) estimates for growth have been attributed to rainbow trout and Atlantic salmon juveniles, whereas moderate to high ($\approx$0.2-0.4) estimates have been determined for adults. McKay et al. (1986) and Kinghorn (1983) also found $h^2$ estimates for older rainbow trout to increase with age. Hershberger et al. (1990) demonstrated improvements in weight gain of over 60% in net-cage cultured coho salmon in a 10-yr selection and breeding program that involved four generations and two stocks. Significant sire and dam effects have also been well documented in breeding experiments. In several cases, the dam component has been larger than the sire component to explain the variance in body weight (Gunnes and Gjedrem 1978; Refstie and Steine 1978; Gjerde and Gjedrem 1984); the relationship between egg size and the size of the juvenile, mentioned above, is a likely factor in this effect.

The production of triploid animals for sterilization has been practiced in salmonid culture (*see* Utter et al. 1983), and since it is a manipulation of the genetic makeup of the animal it should be discussed under this heading. In general, the induction of triploidy has either not affected growth, or retarded growth in salmonids (Figure 10). The exceptions to this are found in studies by Boulanger (1991) where the triploid fish were found to be twice the size of diploid brook trout after the first year of rearing, and by Myers and Hershberger (1991) where the growth rate of one group of triploid rainbow trout was initially lower than diploid fish, but recovered and was more rapid than diploid fish during a growth stanza of 63-82 d post ponding. The second group of triploid fish in that latter study were initially larger than diploid fish, but ended up smaller than the diploid animals at the end of the 102-d experiment. As Figure 10 shows, the overall growth performance between diploid and triploid fish in that study were not very different. In contrast to these studies, similar growth rates between diploid and triploid fish have been reported for rainbow trout (Oliva-Teles and Kaushik 1987, 1990; Benfey 1988; Sutterlin and Collier 1991), Atlantic salmon (Sutterlin and Collier 1991), and coho salmon in fresh water (Johnson et al. 1986). Lower growth rates in triploid salmonids have been reported for rainbow trout (Lincoln and Hardiman 1982; Thorgaard et al. 1982; Solar et al. 1984), and coho salmon in fresh water (Utter et al. 1983) as well as in salt water (Johnstone et al. 1991).

### *Rearing Density*

As rearing density in salmonid culture is increased, the general response is for growth to decline (Figure 11). In full production scale experiments, the inverse relationship between rearing density and growth has been shown in chinook salmon (Mazur 1991) and rainbow trout (Papoutsoglou et al. 1987). The reader is also referred to Chapter 6 in this book. Those results are supported by laboratory studies, although some studies have shown no effect of rearing density on either growth or other performance characteristics such as mortality and disease resistance. These differences may be due to the unique combination of the absolute densities imposed in each study, and the response of the particular species to those conditions (*see also* Chapter 6).

There are several recommended densities for the optimum rearing of salmonids. As a guideline for Canadian government hatcheries, Shepherd (1984) recommended a maximum density of 16 $kg/m^3$ for coho salmon at 13°C (quoted by Blackburn and Clarke 1990). Piper et al. (1982) recommend a density index ($DI = lb \cdot ft^{-3} \cdot in^{-1}$ body length) of 0.5 for salmonids.

Wedemeyer (1976) recommended rainbow trout to be held at 13-61 $kg/m^3$ and coho salmon to be held at 16 $kg/m^3$ after handling to enhance recovery from that stressor. These guidelines, which have been largely developed on data from production facilities, might be considered to be low by the body of evidence from experiments that have investigated the relationship between rearing density and performance characteristics such as growth. Most of the data presented below will be higher, in some cases much higher, than these guidelines (*see* Table 4). One reason for this discrepancy might be the fact that production fish do not usually distribute themselves evenly in a rearing container; thus the effective rearing space is smaller, and actual rearing density higher, than what one would calculate by simply dividing the biomass in the rearing unit by the entire

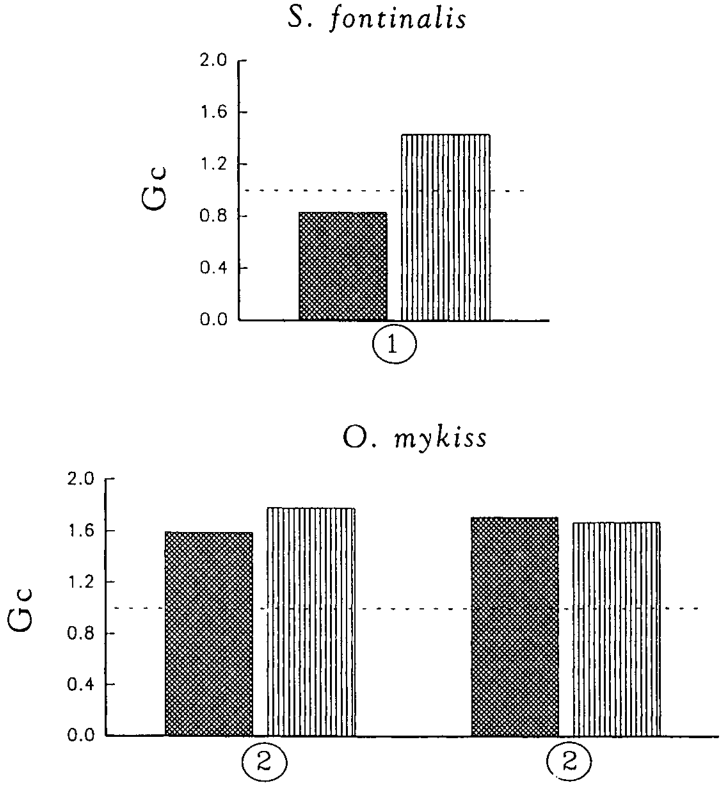

Figure 10. A series of graphs adapted from two studies comparing the growth of diploid and triploid fish. Plots are arranged by species with clusters of bars showing mean growth constant of diploid (crosshatched) and triploid (vertical lines) fish within one study. The dotted lines show a Gc of 1.0. References are given as number within circles below bars. Reference numbers are as follows: 1 Boulanger 1991; 2 Myers and Hershberger 1991.

volume. While there are numerous studies showing that high rearing densities have resulted in lower growth rates in salmonids, there are almost an equal number of studies that have shown densities not to affect growth rates (Table 4). The Arctic charr holds a singular place in the study of cultured salmonids in consistently showing a positive correlation between rearing density and growth. The rearing densities in which they can be reared with good growth rates are also the highest among the commercially reared salmonids. The health and general condition of Arctic charr would probably be adversely affected by rearing densities that would be considered normal for other salmonids, but extremely low for Arctic charr. Some of the key factors that can affect the relationship

**Table 4**

Selected data on the effects of high rearing density on growth of several salmonid species.

| **Species (Initial Weights)** | **Initial Density Range Tested** | **Optimum Density or Highest Growth Rate** | **Effect of High Density on Growth** | **Reference** |
|---|---|---|---|---|
| Arctic charr fry | 25-250 fry/l | 70-250 fry/l | positive | Wallace et al. (1988) |
| Arctic charr (5.5 g) | 5.3-37 kg/$m^3$ | 37-72 kg/$m^3$ | positive | Wallace et al. (1988) |
| Atlantic salmon | 11.4-92 kg/$m^3$ | | none | Soderberg and Meade (1987) |
| Coho salmon | 6.1-26.2 kg/$m^3$ | | none | Blackburn and Clarke (1990) |
| Atlantic salmon | 35-125 kg/$m^3$ | | none | Kjartansson et al. (1988) |
| Rainbow trout | 24-172 kg/$m^3$ | | none | Pickering and Pottinger (1987) |
| Brown trout | 18 and 123 kg/$m^3$ | | none | Pickering and Pottinger (1987) |
| Coho salmon | 1.1-3.0 kg/$m^3$ | lowest | negative | Fagerlund et al. (1981) |
| Rainbow trout (22 g) | 3.3-13.2 kg/$m^3$ | | negative | Trzebiatowski et al. (1981) |
| Lake trout | 27.3-221.4 kg/$m^3$ | below 110.3 kg/$m^3$ | negative (but n.s.) | Soderberg and Krise (1986) |
| Brook trout | 30-120 kg/$m^3$ | lowest | negative | Vijayan and Leatherland (1988) |
| Rainbow trout | 134-277 kg/$m^3$ | lowest | negative | Leatherland and Cho (1985) |
| Rainbow trout | 31-312 kg/$m^3$ | below 250 kg/$m^3$ | negative | Papoutsoglou et al. (1987) |

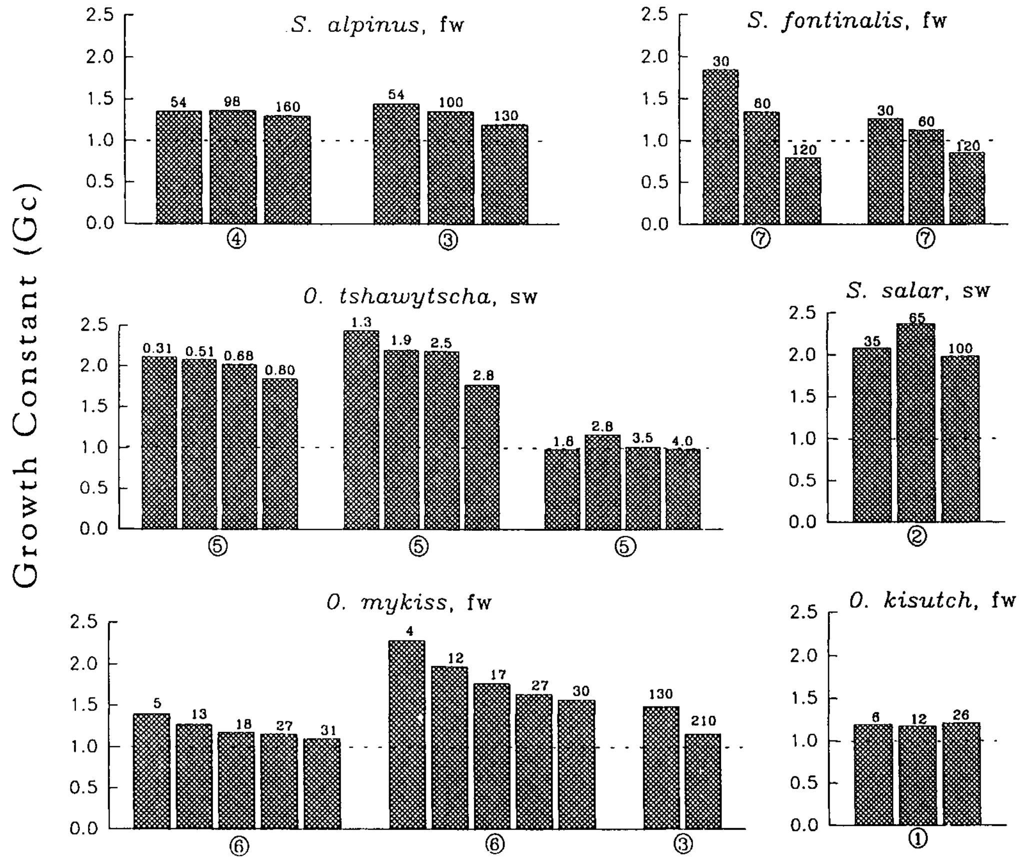

Figure 11. A series of graphs adapted from a number of studies of the effect of stocking density on growth. Plots are arranged by species and water salinity with clusters of bars showing the mean growth coefficients (Gc) of fish reared at a variety of densities. The stocking density associated with each mean is given on the top of the bar (kg/m$^3$) and dotted line shows a Gc of 1.0. The references are given as numbers within circles below the bars. References numbers are as follows: 1 Blackburn and Clarke 1990; 2 Kjartansson et. al. 1988; 3 Leatherland and Cho 1985; 4 de March 1990; 5 Mazur 1991; 6 Papoutsoglou et. al. 1987; 7 Vijayan and Leatherland 1988.

between rearing density and growth include: the age, size and species of fish and their behaviour in response to crowding; feeding level; water quality; and hormonal profile.

The causes for the effect of density on growth in cultured salmonids is still equivocal. While it is tempting to assume that higher rearing densities induce a stress response, at some energetic cost, and that such a stress causes an attenuation of growth, the source of the stress is not clear in most studies. Possible causes of that stress include behaviour and water quality. Investigators in most cases have maintained water quality within acceptable limits for salmonid culture, and some have ascribed the lack of rearing density effects to the maintenance of good water quality (Blackburn and Clarke 1990; Poston and Williams 1988; Kjartansson et al. 1988). Many investigators, therefore, have attributed the possible stress and the associated effects on performance to behavioural causes. The source of the behavioural factor could lie in the dominance hierarchy that is established in populations of fish. This could be manifested in the simple limitation of consumed food by smaller fish, because most of the feed would be eaten by the larger, more dominant fish. A reduction in food consumption has been shown in brook trout, along with growth rates and feed conversion efficiency (Vijayan and Leatherland 1988), and in Atlantic salmon (Fenderson and Carpenter 1971; Refstie and Kittlesen 1976). Wedemeyer (1976), however, showed a normal feeding level in rainbow trout at high rearing densities.

Although Blackburn and Clarke (1990) did not see a significant change in the weight-frequency distribution among 0-age coho salmon reared at three densities, several studies have shown a skewing of the weight-frequency distribution toward the smaller fish with increases in rearing density (Fagerlund et al. 1981, 1983; Schreck et al. 1985). At higher rearing densities, the observation that the big fish get bigger and that an increasing number of fish do not grow as fast as the dominant fish, suggests the possibility that in those studies where substantial mortalities occurred, the smaller fish probably suffered the greatest losses. This may have been a significant factor masking what may otherwise have been significant downward shifts in the mean individual weight of the fish reared at high densities in some studies, particularly those that show no effect of density on growth rates. Related to the food limitation is the possible increase in aggressive behaviour in most cultured salmonids that accompanies higher rearing densities. Given adequate water quality, the possible increase in aggressive behaviour associated with a higher likelihood of interactions between individuals could be the primary cause of the stress at higher rearing densities. The exception to this generalization is the Arctic charr, which is discussed below.

While some studies have shown plasma cortisol, the stress-related hormone, to increase with rearing density (Barton et al. 1980; Fagerlund et al. 1981; Klinger et al. 1983; Pickering and Stewart 1984; Leatherland and Cho 1985), others have shown cortisol to either not change (Laidley and Leatherland 1988), or to decrease with rearing density (Vijayan and Leatherland 1988). Cortisol has been shown to transiently increase and then return to baseline levels within 6 d in brown trout and within 10 d in rainbow trout after an imposed increase in density (Pickering and Pottinger 1987). This evidence, which shows that stressed states in crowded fish may exist while showing baseline cortisol concentrations, suggests that cortisol is probably not always a reliable indicator of stress in fish in this context. Vijayan and Leatherland (1988) showed that cortisol was correlated with growth rate and body size in brook trout that were reared at 30, 60, and 120 kg/m$^3$; they suggested that cortisol was a more reliable indicator of metabolic rate than generalized stress. Since cortisol has been shown have the catabolic effect on lipids to produce quick energy, possibly to cope with stressful conditions, it can be seen to support the observed positive correlation of cortisol and growth rates in their study. This mobilization of stored energy in the lipids would have a protein-sparing effect in fish that would present an advantage to the animal if feed was being limited through increased competition at higher densities.

Thyroid hormones have been shown to be inversely correlated with rearing density in coho salmon parr and smolts (Schreck 1981) and in brook trout (Vijayan and Leatherland 1988). This may be one causal factor where decreased growth has been associated with high rearing density, as $T_4$ has been shown to have anabolic effects (Higgs et al. 1982; Leatherland 1982).

Wallace et al. (1988) showed that in Arctic charr fry that have just initiated feeding, growth in the fry reared at densities of 70 to 250 fry/l was higher, and the mortality lower, compared to those reared at 25 to 50 fry/l. In fingerlings, they found the best growth to be 37-72 kg/m$^3$ and the lowest growth in fish reared at 5-10 kg/m$^3$. In the larger fish (5.5 g and 16 g, starting mean weights) they studied showed a positive correlation between growth and rearing density, where the maximum densities were 130 and 170 kg/m$^3$. Those authors speculated that the cause for such a positive correlation was the suppression of aggressive behaviour and an increase in schooling behaviour at high rearing densities; they draw an analogy to the eel in which the dominance hierarchy has been shown to be broken down at densities above 25 kg/m$^3$ (Seymour 1984). The densities at which the best growth rates that have been observed in Arctic charr clearly overlap with those densities at which other salmonids have shown a depression of growth rates. It would be valuable in future research to describe the relationship between physiological stress and the behavioural characteristics of the fish at various rearing densities. Does the social hierarchy breakdown as densities increase above a certain point? Is physiological stress induced when that point is crossed? Why is aggressive behaviour suppressed at high densities in Arctic charr, but apparently enhanced in other salmonids? Answers to these questions for the major species of salmonids cultured would contribute greatly to our quantitative understanding of our intensive culture system.

Several studies have shown that the polyculture of Atlantic salmon and Arctic charr has resulted in the mitigation of the negative effects of crowding, which would be observed in the monoculture of Atlantic salmon. Holm (1989) demonstrated that the intensive co-culture of these species resulted in significantly higher growth rates in the Atlantic salmon, compared to the monoculture of that species. There was evidence of lower aggressive behaviour and a greater dispersion of individuals. Those results were confirmed by the study of Nortvedt and Holm (1991), which attempted to discern the contribution of shading or the thinning of conspecifics by the other species to the increased growth in the Atlantic salmon. The Arctic charr tended to gather around the centre of the tanks, whereas the Atlantic salmon distributed themselves more in the corners and along the sides of the container. They concluded that shading played a role in the reduction of interaction among individuals in the Atlantic salmon population, and they speculated that this effect might be more pronounced at higher densities. The growth of Arctic charr was not significantly affected by this co-culture. The apparent unique response of Arctic charr to crowding in culture conditions, as discussed above, probably make this species an ideal candidate for this strategy of managing high density rearing of two salmonid species. Jobling and Reinsnes (1986) studied the effects of social interactions in Arctic charr on growth. They found that growth in a population was suppressed by the introduction of faster-growing individuals. Visual contact was not the cause for this effect, and individuals grown in isolation showed a higher growth rate than those grown in groups; those higher growth rates, however, were lower than those from groups at optimal densities.

It is clear that in this most basic of considerations in the intensive culture of fish, there is a great lack of understanding on which to base plans for loading tanks with fish. Rearing density is one of the most important parameters in the economic planning or analysis of intensive aquaculture systems. How far can one push the relationship between the particular fish species and life style with the rearing container and water quality conditions? The answers can only be found in further research about the basic nature of the animal to be cultured, combined with comprehensive applied studies that measure production parameters such as growth, health, and feed conversion efficiency as well as physiological parameters such as the hormonal profile and stress indicators in salmonids reared under various densities, in conditions that can be extrapolated to actual production systems.

### *Other Factors*

The relationship between sexual maturity and growth rate in salmonids has been discussed in the literature, mostly pertinent to the subject of precocious sexual maturation (*see* Thorpe et al. 1983; Thorpe 1986). As Figure 12 shows, the growth rate of maturing adult salmonids is retarded by sexual maturity, while that of precociously maturing juveniles is enhanced. This relationship should serve as a warning to selection programs that focus on enhancing high growth rates, as this could lead to a decline in the age at first maturity, or precocious sexual maturation. Although it may seem odd to give this topic this apparent cursory treatment here, one of the focuses of this chapter is the effects that various factors have on growth rates in salmonids; not how growth rates, among other factors, play a role in the normal or precocious sexual maturation of salmonids. The decline in somatic growth rates of salmonids with sexual maturation has been shown by several workers (Taylor 1989; Lamont 1990; Jobling and Baardvik 1991). This is caused primarily by several factors: the increase in energy demands by the developing gonadal material; the reduction in food intake (Rowe and Thorpe 1990); and the shunting of energy from somatic growth to gonadal development. The large amount of yolk material in developing eggs, for example, incorporates fat and protein from eaten food, as well as from body tissues (*see* Tveranger 1986). Under conditions of culture,

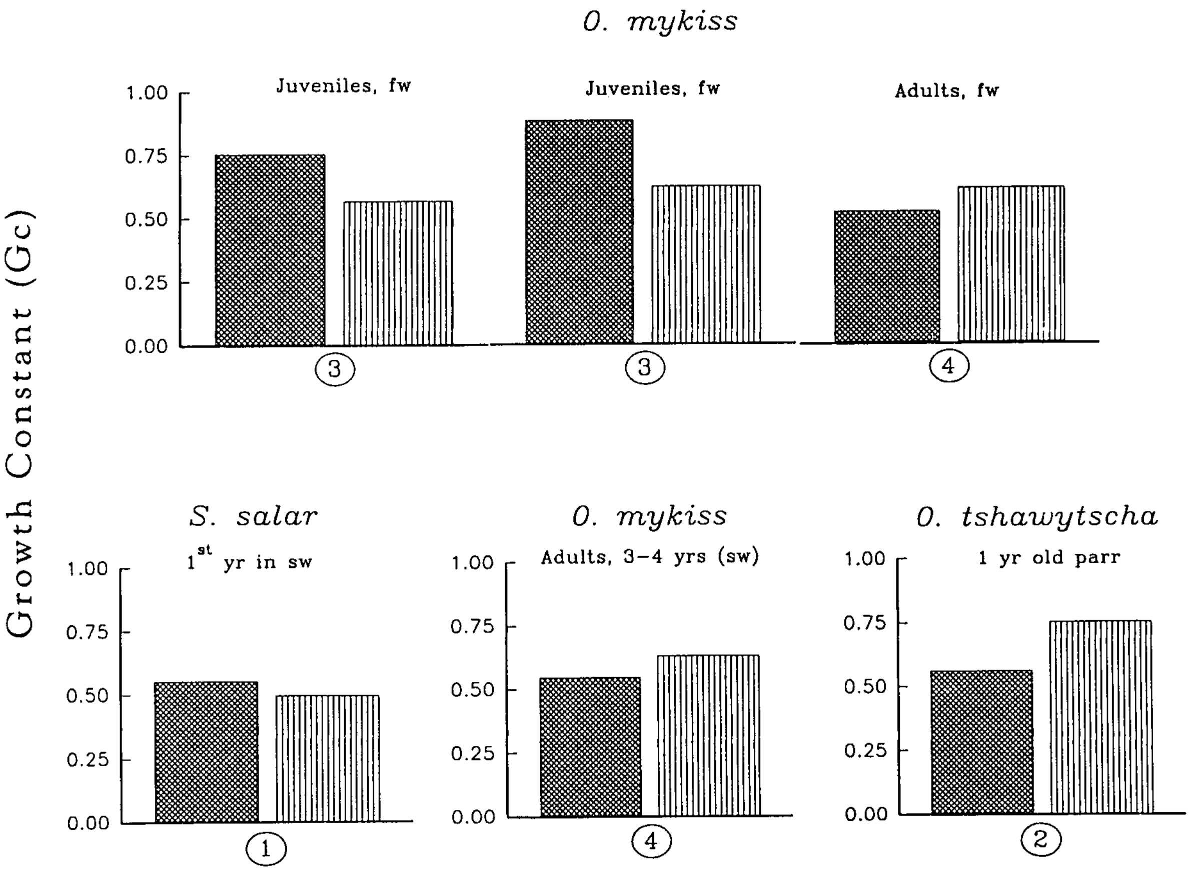

Figure 12. A series of graphs adapted from studies of maturation in fish. Plots are arranged by species with brief descriptions of fish age. Pairs of bars show mean growth constant (Gc) of fish that matured (cross hatched bars) or did not mature (vertically lined bars) during or shortly after the experimental period. The references are given as number within circles below the bars. References numbers are as follows: 1 Erikssen and Lundqvist 1982; 2 Iwama and Bernier 1992 (unpubl. data); 3 Lamont 1990; 4 McKay et al. 1986.

however, this is of little economic consequence as maturing fish in brood stocks are not destined for market.

The administration of growth hormone, somatotropin, or their analogs, by injection, immersion, implants, or through the feed, have resulted in improved growth in several salmonid species (Figure 13). Recombinant bovine somatotropin (RBST) has been shown to dramatically (in the order of 20-40% over sham controls) stimulate growth in coho salmon (Down et al. 1989; McLean et al. 1990), and rainbow trout (Schulte et al. 1989). Agellon et al. (1988) also showed a 100% increase in growth rate in rainbow trout injected weekly with recombinant fish growth hormone. Boeuf et al. (1990) has demonstrated growth enhancement in juvenile Atlantic salmon given bovine growth hormone in a cholesterol implant. It is interesting to note that the concentrations of growth hormone in the plasma and pituitary were as much as five-fold higher in coho salmon stunts, compared to other smolts (Bolton et al. 1987). The investigators speculated that there must have been deficiencies in either the receptor populations, or in the mediating systems that effect the growth-promoting processes.

It is both impossible to ignore the cyclical rhythm of fish growth, and tempting to explain it with lunar cycles or the effect of tides. In spite of a fixed 12h:12h (light and dark) photoperiod, Farbridge and Leatherland (1987) showed a cycling of rapid and slow growth in rainbow trout, with each phase lasting about 2 wk. The peaks, fitted with algorithm, were associated with the new and full moons, and changes to this cycling pattern occurred in coincidence with the spring equinox. These investigators also provided evidence, from glycine uptake rates by scales *in vitro* and the correlation of those rates with RNA:DNA ratios in liver and muscle, to suggest that the cycling of growth rates in the intact animal were probably due to real tissue elaboration, rather than fat deposition or water shifts. Such cycling of growth rates have also been shown by Wagner and McKeown (1985) in rainbow trout, and by Jobling (1987) in Arctic charr. While the mechanisms for this phenomenon are far from clear, it is possible that appetite-regulating hormones may cycle to an endogenous rhythm that may be tied to lunar or tide cycles (*see* Jobling 1987; Farbridge and Leatherland 1987 for discussion).

Related to seasonal effects are the effects of photoperiod on growth. By manipulating photoperiod regimes, Clarke and Shelbourn (1986) increased growth rate in coho salmon in both fresh water and seawater by delaying the natural season by 30 d. Saunders and Harmon (1990) found that a 18h light:6h dark photoperiod regime gave the best growth in juvenile Atlantic salmon, and that the duration of exposure to this regime, as well as the time of year in which this was done, affected the photostimulation of growth. This extension of daylength for a period of 3 mon gave favourable long-lasting stimulation of growth, compared to a 2-mon exposure period. It is possible that increased growth hormone (Komourdjian et al. 1976, 1989; Bjoernsson et al. 1989) may be mediating the stimulation of growth with these manipulations of photoperiod.

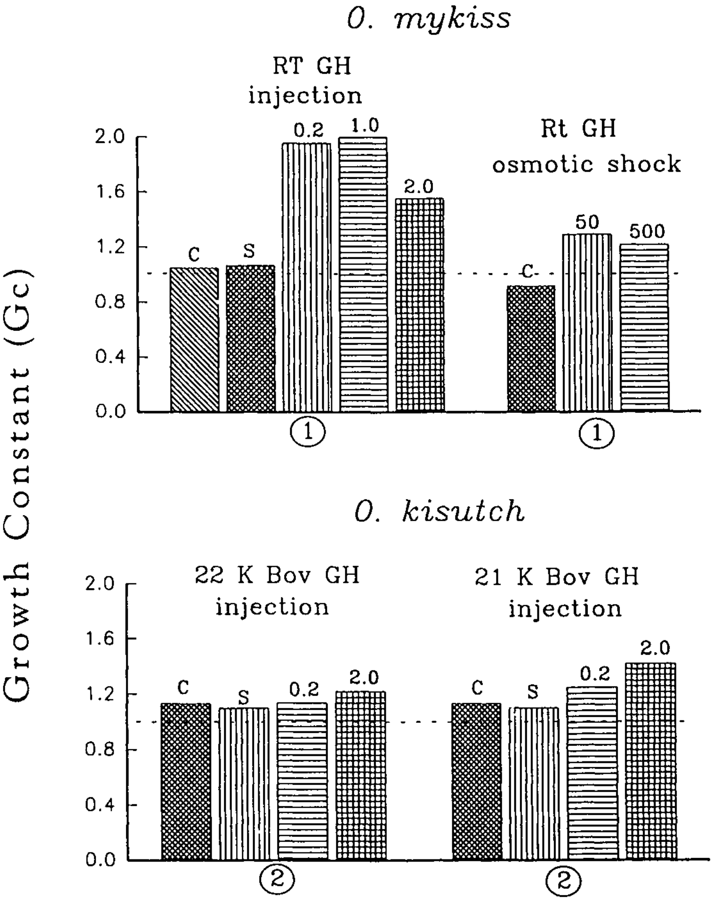

Figure 13. A series of graphs adapted from two studies of the effects growth hormone administration on growth. Plots are arranged by species with brief descriptions (GH=growth hormone, RT=rainbow trout, Bov=bovine). Clusters of bars show mean growth constant of different treatments within one study. Bars labelled C and S represent control and sham injected fish respectively while numbers represent the μg/kg (for injections) or μg/l (for bath treatments). The dotted line shows a Gc of 1.0. The references are given as number within circles below the bars. The reference numbers are as follows: 1 Agellon et al. 1988; 2 Down et al. 1989.

## CONCLUDING REMARKS

Fish growth is one of the vital parameters in aquaculture, whether it be for the commercial production of a food fish or for the enhancement of natural populations. This review has attempted to describe some of the intrinsic and extrinsic factors that can influence growth in salmonids, with particular attention to aquaculture. Those effects among various data sets were shown by the use of a simple growth model, which was designed for predictive convenience. Of the environmental factors, temperature has the potential for exerting the greatest influence on growth rates in fishes. While optimum temperatures for culture have been determined for most salmonid species, the influences of fluctuating temperatures, as occur in nature, on growth rates in fishes should be investigated further. Although there is a good knowledge base about the basic nutritional requirements of salmonids, more research is needed about the possibilities of using alternate sources of protein and how they, along with other minor ingredients, affect the short-term and long-term health of the individual fish as well as the population. Feeding strategies should be refined for economy, and to reduce potential impacts of intensive aquaculture on the environment. Further research is needed to more fully describe the physiology of compensatory growth, including the possibility that potential stress due to the periods of starvation might be adversely affecting the fish in the long term. The effects of water salinity on growth appear to depend on the life stage and life cycle of the species under culture. Further research on assessing the energetic cost of ionic and osmotic regulation may provide potentially valuable information that could improve the efficiency of culture operations. While the general trend for increasing densities to depress growth rates in salmonids seems to be consistent among most of the cultured species, the mechanisms by which this takes place has not been well described. The preference for Arctic charr for high densities places them in a relatively unique category and invites further study.

Intrinsically, the genetically based propensity for growth in all cultured salmonid species must be explored and quantified further. Although that information may allow the manipulation of growth without the less desirable use of hormones or synthetic agents, the application of molecular techniques for the direct manipulation of the genome must be evaluated carefully, as the possibility exists for genetically altered fish to affect natural stocks. While salmonid culture may enjoy the benefits of having as its subjects the most intensively studied families of fishes, large gaps exist in the most fundamental consideration of intensive animal production. The study of fish growth presents an unbounded area for basic, or "not yet applied", research. Research into fish growth as it applies to various aspects of aquaculture should be conducted with the awareness of the fate of the product, whether it be for direct consumption as a food commodity or whether it will be planted in the natural environment to interact and breed with wild populations.

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## Chapter 8

# SMOLT PRODUCTION

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## INTRODUCTION

Hatcheries for production of salmonids were well established during the latter part of the last century (*see* Chapter 1). For the most part, these pioneering efforts concentrated on the production of fry and fingerlings for release into streams. During the last four decades, large-scale hatchery programs involving rearing of smolts for release have been established in Europe, North America and Asia. More recently, a large international salmon farming industry has come to depend upon the production of millions of smolts for stocking into netpens and rearing to a marketable size.

"As the winter's chill is taken off the waters by the warm sun of spring, the fry grows and grows, escaping all kinds of dangers, and increasing in weight and strength, till it is gratefully recognized by the juvenile angler as the little parr, clad in a very gay livery, and which nobody believed, till lately, would ever become a salmon. An interesting episode occurs when the little fish attains the first year of its age — one-half of the shoal becoming smolts, eager for change of scene; the other half remaining in the parr state for a year longer" (Anonymous 1861).

When this description of juvenile development was written, naturalists were just beginning to understand the life cycle of Atlantic salmon (*Salmo salar*). More than a century later, we now recognize that the transformation of the stream-dwelling parr to the silvery smolt entails many behavioral, morphological and physiological changes that prepare juvenile salmonids for their feeding migration to the sea (Hoar 1988).

This understanding has been achieved through considerable experimentation, particularly with Atlantic and coho salmon (*Oncorhynchus kisutch*) as is evident in several symposia on salmon smolting (Bern and Mahnken 1982; Thorpe et al. 1985; Hansen et al. 1989; Saunders et al. 1994*a*). The emphasis on these two species is due to both their economic importance and their well-defined parr-smolt transformation, which is amenable to study.

However, the classic description of smolting as developed from studies of Atlantic and coho salmon does not pertain to all anadromous salmonids because there is considerable variation among species (Hoar 1976; McCormick and Saunders 1987; McCormick 1994; Clarke and Hirano 1995). Smolts vary not only with respect to the size and age at which they are able to thrive in seawater but also in the seasonal environmental cues which synchronize their development. On the one hand, pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon enter the ocean soon after emergence to grow for only a year or more; on the other, Arctic charr (*Salvelinus alpinus*) enter the sea for a brief period of a few weeks or months during the summer.

In this chapter, we review the biology of the parr-smolt transformation in the major anadromous species that are under cultivation, indicating the features that they share and the differences among them. These differences have led to varying techniques for rearing them.

The first salmon hatchery in the U.K. was established in 1868 (*see* Chapter 1). Salmon hatching techniques were introduced to Japan in 1876 and by 1888, the Chitose Central Salmon Hatchery was established near Sapporo, Hokkaido (Kaeriyama 1989). The first salmon hatchery in North America was built by Samuel Wilmot at Newcastle on Lake Ontario. Upon Wilmot's recommendation, in 1866 the Government of Upper Canada, by an order-in council, reserved Wilmot's Creek for the natural and artificial propagation of Atlantic salmon (Dunfield 1985). Wilmot was later superintendent of fish culture for the federal government from 1876 to 1895 and established 15 hatcheries across Canada. The Craig Brook salmon hatchery near East Orland, Maine was established in 1871 and it is still open today.

The first salmon hatchery in western North America was built in 1872 on the McCloud River in California under the direction of the U.S. Fish Commission. It incubated chinook salmon (*Oncorhynchus tshawytscha*) eggs to the eyed stage and then shipped them by stage coach and train across the U.S. for stocking rivers on the Atlantic coast. Numerous hatcheries were then established in Washington and Oregon beginning in 1877 (Nash 1995). The first salmon hatchery in British Columbia was built at New Westminster, near the mouth of the Fraser River in 1884 and by 1910 there were eight such facilities (Gough 1991). The first of many Alaskan hatcheries was built on Kodiak

Island in 1891 and by 1901, one with a capacity of 110 million eggs was built on Revillagigedo Island near the southern tip of the Alaska panhandle. The hatcheries in British Columbia and Alaska concentrated on the production of sockeye salmon (*Oncorhynchus nerka*). By 1929 there were 72 hatcheries from California to Alaska; the cumulative releases of fry up to 1928 were in excess of 12 billion (Wahle and Smith 1979).

Between 1929 and the early 1940s, all but three of the 18 U.S. federal salmon hatcheries were closed or turned over to state governments. The hatcheries in British Columbia, Alaska and California also were closed but most hatcheries operated by the States of Washington and Oregon remained in operation.

Decline in support for hatcheries came with the realization that they were contributing little if anything to the fisheries. The standard practice of the early hatcheries was to release fry soon after the eggs hatched, when the yolk sac had been absorbed (Wahle and Smith 1979). In the absence of scientific knowledge of basic husbandry, staff of the early hatcheries learned by trial and error. Improvement of techniques for producing smolts for release evolved gradually from the early empirical practices. Over time, it was learned that larger, older juveniles had a much higher rate of survival to adulthood than did fry. A major breakthrough in this area was the introduction of effective manufactured diets in the early 1960s which greatly improved growth and health of presmolt salmon (Cleaver 1969). Current fish husbandry techniques based on systematic investigation of salmon biology have evolved in concert with the expansion of hatcheries for stock restoration since the 1950s and 1960s and more recently with the growth of commercial salmon farming.

There was a resurgence of hatchery construction in the Columbia River basin in the 1940s following construction of the Grand Coulee Dam that prevented Pacific salmon from migrating to spawning areas in the upper portions of the river. Numerous additional hydroelectric dams constructed throughout the lower basin resulted in a number of new hatcheries being established with the aim of restoring the wild stocks caused by habitat alteration (Wahle and Smith 1979). Similarly, large-scale hydroelectric development on major salmon rivers in Sweden after World War II stimulated the construction of hatcheries rearing Atlantic salmon smolts to compensate for lost natural production (Lindroth and Larson 1985). With the advent of improved techniques for production of smolts, many hatcheries in Alaska were re-opened beginning in the 1950s and in British Columbia in the late 1960s and early 1970s.

## BIOLOGY OF THE PARR-SMOLT TRANSFORMATION

An understanding of the physiological basis for the parr-smolt transformation (or smoltification) is required for the effective operation of salmon hatcheries. Whether they are producing smolts for release in support of fisheries or for transfer to netpens for growout, hatcheries must provide coordinated environmental cues to ensure that smolt development is synchronized for a successful transition to seawater.

### *Changes Associated with Smolting*

*Life History and Anatomical Changes* (*see also* Chapters 2 and 3). Changes in body form and colouration are most obvious in species such as the Atlantic salmon (McCormick et al. 1985) and the coho salmon (Gorbman et al. 1982; Winans and Nishioka 1987). In these species, the deep-bodied, cryptically coloured stream-dwelling parr becomes a slender, silvery smolt with darkly pigmented margins of the dorsal and caudal fins. The development of silvering is caused by the deposition of guanine and hypoxanthine in two layers beneath the scales and deep in the dermis (Markert and Vanstone 1966; Johnston and Eales 1967).

Atlantic salmon occur in rivers flowing into the northeast Atlantic from Spain and Portugal north through France, the U.K., Ireland, Norway, Sweden, Finland, Russia, across to Iceland, Greenland, and down through rivers on the western side of the Atlantic Ocean through Canada and the U.S. as far south as Connecticut. In some European rivers, Atlantic salmon may smolt at age 1+ (i.e., S1), but most are two years old. Smolts in eastern North America are rarely under two years old; four- and five-year-old smolts are not uncommon in the northern part of the range (Power 1961). Atlantic salmon smolts range from 125 to 180 mm fork length, depending on age and stock.

As with Atlantic salmon, brown trout (*Salmo trutta*) exist in both anadromous (often termed sea trout) and resident forms. Anadromous and non-anadromous fish of both sexes can occur in the same river and are apparently the same genetic stock (Hindar et al. 1991). Smolts develop after two to six years and range in size from 110 to 200 mm fork length. The downstream migration occurs in spring and adults spend from one to three years at sea before returning as mature spawners (Økland et al. 1993; L'Abee-Lund 1994).

All species of Pacific salmon are propagated in hatcheries for release in order to support commercial and recreational fisheries. Chinook and coho salmon are also produced for stocking netpens in commercial salmon farms.

The masu salmon (*Oncorhynchus masou*) occurs only in Asia, specifically in Japan, eastern Korea and northward to the Sea of Okhotsk. In northern areas such as the Island of Hokkaido, it is mainly anadromous but in the southern part of its range it completes its life cycle entirely in the rivers as "yamame". The stream-dwelling juveniles are deep-bodied and have prominent parr marks. The smolts are readily distinguished by a loss of parr marks, development of a silver colour and a more slender body form. In Hokkaido, the peak downstream movement of masu salmon smolts occurs during the first half of May of the second spring (Kato 1991). The smolts average 110-130 mm fork length. Many rapidly growing underyearling males become sexually mature in autumn; they do not become smolts in the following spring but remain in the river (Aida et al. 1984). The amago salmon (*O. rhodurus*) is closely related to the masu salmon and is considered to be a subspecies by some authors (Numachi 1984; Robins et al. 1991). The amago salmon has a very limited distribution in rivers along the eastern coast of central Honshu Island and on nearby Shikoku Island, Japan (Kato 1991). Juvenile amago salmon grow in streams during the first year. Many males become sexually mature in autumn of the first year and are not able to acclimate to seawater, while a variable proportion of the larger sexually immature fish become smolts in late November and December (Nagahama et al. 1982). Closely related to the amago is the "biwamasu", a lake-dwelling form from Lake Biw" on southwestern Honshu Island, Japan. Fry of the biwamasu become partially silvered during their first spring and migrate downstream into Lake Biwa where they grow for three to five years until they return upstream to spawn. When held under pond conditions, biwamasu grow more slowly and exhibit less intense body silvering and darkening of the outer margins of the dorsal fin than do amago (Fujioka 1987).

The coho salmon is most common along the west coast of North America where it is found from California to Alaska; on the Asian coast it occurs from the Bering Sea south to Sakhalin Island and the northern part of Hokkaido Island in Japan (Sandercock 1991). Juvenile coho salmon usually spend at least a year in fresh water, often in small coastal streams. The seaward migration of smolts occurs from late April to June. As with the masu, the stream-dwelling parr are cryptically coloured with prominent parr marks while the smolts are silvery.

The chinook salmon occurs in medium and large rivers from California to northern Hokkaido (Healey 1991). There are two major juvenile life history forms: "ocean-type", which enter the sea as underyearlings, and "stream-type", which spend one or more years in fresh water. Ocean-type juveniles migrate downstream either soon after emergence as fry or after several months as fingerlings and remain in low salinity waters of estuaries for one or two months until they reach a size of 70 mm fork length. Subsequently, they move offshore. The stream-type life history pattern is predominant in Asian and Alaskan populations and occurs also in headwater tributaries, particularly in larger rivers, elsewhere in North America (Taylor 1990; Healey 1991). They reside in rivers for one or more years until they reach a size of 75-125 mm fork length and undertake the seaward smolt migration; unlike the ocean-type juveniles, they do not reside for an extended time in estuaries, but disperse into marine waters. Chinook fry have large parr marks. Unlike coho, ocean-type chinook do not undergo as distinct a transformation from parr to smolt while in fresh water; instead they gradually become more silvery as they grow in estuarine areas. In contrast, the stream-type chinook smolt is readily distinguished from the parr stage by its silvery colour, elongated shape and darkly pigmented margins of the dorsal and caudal fins.

Sockeye salmon are found along the North American coast from the Klamath River in California to the Yukon River in Alaska and along the coast of Asia from the northern Bering Sea south to the northern shore of the Sea of Okhotsk (Burgner 1991). Sockeye salmon exhibit a variety of life history patterns. At one end of the spectrum is the kokanee, a permanent freshwater resident form of sockeye salmon that may occur in sympatry with anadromous populations or in lakes which are not accessible from the sea. Kokanee are found naturally in lakes from Idaho around to Hokkaido but their range has been extended considerably by introductions. Juvenile sockeye usually reside in lakes for at least one or two years before migrating seaward as smolts from April to June. However, in some river systems without lakes, there are "ocean-type" populations of sockeye salmon that enter the sea as underyearlings and "river-type" populations that inhabit river channels for at least one year (Birtwell et al. 1987; Wood et al. 1987). Sockeye fry have short parr marks and black spots on the back. The pelagic feeding stage in lakes has a darker back and the parr marks become less distinct with silvering of the sides. At the time of smolting, they become more silvery and streamlined.

The chum salmon is found along the Pacific and Arctic coasts of North America from California to the Mackenzie River and in Asia from the Lena River in the Arctic south to Korea (Salo 1991). Juvenile chum enter the sea as underyearlings, usually soon after emergence at a size of 35-45 mm. However, in some populations, migration may be delayed and the fry grow to 60 or 70 mm in length in the river or estuary (Sparrow 1968; Mason 1974). In the Amur River, juvenile chum salmon may grow to a size of 90 mm. Although chum salmon fry have small parr marks, there is no distinct morphological transformation to the smolt stage.

The pink salmon has an extensive range from California to the Arctic coasts of North America and Asia and along the western Pacific coast to Korea on the Japan Sea (Heard 1991). Juvenile pink salmon migrate seaward very soon after emergence from the gravel at a size of 28-35 mm fork length and 130 to 260 mg in weight. Juvenile pink salmon lack typical parr marks; the fry are green on the back and silver on the sides from the time of emergence.

Steelhead trout (*Oncorhynchus mykiss*) are the anadromous form of rainbow trout. They are found in North American rivers from California to Alaska and in Asia, mainly on the Kamchatka peninsula (Burgner et al. 1992). Throughout this range, they co-occur with non-migratory rainbow trout. Juveniles reside for one to five years in fresh water before entering the sea as smolts during late April and early May at sizes ranging from 125-225 mm fork length. Smolts acquire a silvery colour and become more slender.

Most populations of brook trout (also termed brook charr, *Salvelinus fontinalis*) are resident in fresh water although there are anadromous populations in many coastal rivers of northeastern North America (Naiman et al. 1987). In northern latitudes, brook charr migrations are characterized by spring emigrations of 2- to 4-year-old fish that remain in seawater for only two to four months. In the southern portion of its range, seaward migration is more variable, and often occurs in the autumn.

Arctic charr occur as both anadromous and resident populations in a circumpolar distribution in rivers surrounding the Arctic Ocean. Anadromous populations are usually found north of latitude 60°N. Anadromous stocks of Arctic charr typically migrate to the sea for the first time after three to seven years in fresh water at a length of 170 to 260 mm (Finstad et al. 1989*a*). Smolting is not obvious externally in Arctic charr. Unlike salmon, charr do not over-winter in the sea but usually return to fresh water after as little as a month. Another contrast with salmon is that the upstream migrants are frequently not sexually mature (Johnson 1980).

*Physiological Changes.* An essential part of the parr-smolt transformation is an increase in euryhalinity which allows the smolt to live in salinities varying from soft fresh water to full strength seawater. Although the timing of development of hypo-osmoregulatory capacity differs among species, the physiological mechanisms which prepare salmon for life in the sea are common to all.

The body fluids of salmonids have an osmotic concentration approximately one-third that of seawater. While salmonids are in fresh water, the concentration gradient favours entry of water into the body and loss of salts by diffusion (i.e., they are hyperosmotic to the external water). Most of these movements occur across the gill surface because the rest of the body surface is relatively impermeable. To counter these passive flows, the fish excretes the excess water as a dilute urine and obtains salts from the food as well as by active uptake across the gill surface. Once salmonids enter salt water, the osmotic gradient is reversed (i.e., they are hypo-osmotic to the external water). Loss of water and diffusion of salts into the body are restored by drinking seawater; urine flow is reduced to conserve water and salts are actively excreted across the gill by mitochondrial-rich cells called "chloride cells" (*see* Chapter 2). The excretion of salts is accomplished by means of an enzyme, $Na^+,K^+$-adenosine triphosphatase (ATPase), that is often measured as an indicator of smolt quality (*see* page 545).

### *Hormonal Control of Smolting*

Although changes in many hormones are associated with smolting, the actions of most are not well understood. Only the major hormones associated with smolt development that have been most intensively studied are reviewed (*see* Chapter 2 for more information on the endocrine glands and the hormones they secrete).

*Thyroid Hormones.* Smolting involves a number of developmental changes including growth, body shape, behaviour, pigmentation and an increase in euryhalinity. It is perhaps not surprising then, that the thyroid, which has long been known to have important developmental effects in vertebrates, is involved in smolting. Destruction of the thyroid gland was found to depress growth of juvenile steelhead trout and chinook salmon; normal growth was restored following treatment with thyroxine (Norris 1969). Activation of the thyroid in Atlantic salmon smolts was first described by W. Hoar in 1939 but the more recent discovery of a surge in circulating thyroid hormone levels in coho and masu salmon smolts (Dickhoff et al. 1978; Nishikawa et al. 1979) stimulated renewed interest in the role of the thyroid. Although treatment with thyroid hormones usually has little effect on acclimation to seawater, it does cause the increases in silvering and growth rate usually associated with smolting. Triiodothyronine has been administered in the diet in a number of species to accelerate growth and appearance of smolt characteristics (McBride et al. 1982; Shelbourn et al. 1992; Boeuf et al. 1994*a*). Juvenile coho salmon that are transferred prematurely to seawater become stunted and have a number of differences in hormone function compared with typical smolts (Clarke and Nagahama 1977; Nishioka et al. 1982). The thyroid of stunts appears inactive and plasma thyroid hormone levels are reduced (Folmar et al. 1982). Thyroid hormones are also involved in the olfactory imprinting of smolts which facilitates their return to their natal stream as sexually mature adults (Scholz et al. 1985; Morin et al. 1989).

*Cortisol.* A steroid hormone secreted by the interrenal tissue, cortisol plays a major role as an osmoregulatory hormone in teleosts and is immunosuppressive during stress (Mazeaud et al. 1977; Barton et al. 1985; Barton and Iwama 1991). It has long been known that the interrenal cells are hypertrophied in juvenile Atlantic and coho salmon at the time of smolting (McLeay 1975; Olivereau 1975; Specker 1982). Cortisol treatment assisted regulation of plasma electrolyte concentrations in juvenile sea (brown) trout, Atlantic salmon and coho salmon after transfer to seawater (Richman and Zaugg 1987; Madsen 1990; Bisbal and Specker 1991). Cortisol is known to act directly on the gill to induce differentiation of chloride cells and stimulate increased $Na^+,K^+$-ATPase activity (McCormick and Bern 1989). Cortisol also influences changes in lipid metabolism that occur in smolts, such as the reduction in total lipid and triacylglycerol content of dark muscle and liver tissue (Sheridan 1989).

*Growth Hormone.* Pituitary growth hormone not only promotes growth of juvenile salmon but also increases survival in seawater. Although growth hormone is not considered an important osmoregulatory hormone in most teleosts, it has been shown to facilitate regulation of plasma ion concentrations in juvenile salmonids after transfer to seawater (Komourdjian et al. 1976; Clarke et al. 1977; Miwa and Inui 1985; Bolton et al. 1987; Hirano et al. 1987; Madsen 1990) and to stimulate the development of gill $Na^+,K^+$-ATPase (Richman and Zaugg 1987). Secretion of growth hormone increases following entry of salmonids into seawater. Growth hormone stimulates production of insulin-like growth factor I (IGF-I) in the liver, gill, and kidney, which in turn stimulates an increase in gill $Na^+,K^+$-ATPase (Sakamoto et al. 1993, 1994). Growth hormone also stimulates conversion of thyroxine to triiodothyronine (Leloup and Lebel 1993) and sensitizes the interrenal tissue to adrenocorticotropic hormone (ACTH) (Young 1988). Thus, growth hormone acts in combination with cortisol and thyroid hormones to influence smolt development.

*Sexual Maturation*. Sex hormones secreted by the testes of precocious male parr of juvenile amago, Atlantic, chinook, and masu salmon as well as sea trout are inhibitory to smolt development (Nagahama et al. 1982; Aida et al. 1984; Lundqvist et al. 1986; Thorpe 1987; Dellefors and Faremo 1988; Foote et al. 1991).

### *Environmental Cues Governing Smolting*

*Pacific Salmon and Steelhead Trout*. Pacific salmon can be divided into two groups according to the environmental cues that guide their development to the smolt stage (Clarke 1992). In the first group are chum, pink, and ocean-type chinook salmon that do not require photoperiod cues for growth and development to the time of seawater entry. These species grow to a specific size which is associated with attainment of the capacity for hypo-osmoregulation in seawater as underyearlings. Their relatively small size at seawater entry and insensitivity to photoperiod makes them more compatible with hatchery propagation. In the second group are coho, stream-type chinook and masu salmon as well as steelhead trout. In the latter group, both growth and smolt development are influenced not only by temperature but also by photoperiod. They normally remain in fresh water for a year or more and their growth in fresh water is highly seasonal. Successful hatchery propagation of these species is more complex than for the first group since it requires the coordinated application of appropriate temperature and photoperiod conditions; it is also more expensive, because of the larger size of the smolts.

Water temperature has a strong effect on growth rate (*see* Chapter 7) and thus influences the time at which juveniles reach the body size required for smolt development. In this way, temperature can determine not only the year but also the season at which smolt development occurs. Kubo (1965) reported that presmolt masu salmon transferred to elevated water temperatures of 9-14°C in early March became smolts earlier than those held at ambient temperatures of 4-6°C. Juvenile coho salmon held in the laboratory at 10°C exhibited maximal levels of gill $Na^+,K^+$-ATPase one month earlier than those held at 6°C (Zaugg and McLain 1976). Ocean-type chinook juveniles held in the laboratory at 17°C reached maximal hypo-osmoregulatory capacity in seawater about two months earlier than those held at 9°C (Clarke and Shelbourn 1985). This effect of temperature was not strictly a function of body size, because smolts reared at high and low temperature exhibited maximal hypo-osmoregulatory capacity in seawater at different sizes. Seasonal temperature cycles are known to affect smolt development in yearling coho salmon. Accumulated temperature units in a coastal stream during the month of April explained 76% of the variation in median date of migration for wild S1 coho salmon smolts and 80% for S2 smolts (Holtby 1988). Clearcut logging of the watershed increased stream temperatures and advanced the median day of migration by about 10 days. Coho salmon hatcheries in Puget Sound, Washington with a seasonally fluctuating water temperature obtain higher rates of adult returns than do those with a relatively constant temperature during spring (Olson 1978). Similarly, juvenile steelhead trout held on a seasonal temperature cycle of 6.9-18.6°C exhibited greater migratory behaviour and a more pronounced elevation of gill $Na^+,K^+$-ATPase activity than those held at a constant temperature of 12°C (Zaugg and Wagner 1973; Wagner 1974).

Temperatures must be sufficient to permit growth of juveniles to smolt size but not so high as to inhibit acclimation to seawater. Usually, temperatures in the 10-14°C range are most beneficial during the season of smolt development and migration. At 20°C, juvenile coho salmon exhibit only a transitory increase in gill $Na^+,K^+$-ATPase activity (Zaugg and McLean 1976). Acclimation to seawater is impaired at temperatures above 16-17°C.

Photoperiod provides important seasonal information and is the main environmental cue that synchronizes the annual cycle of growth and smolting in species such as coho, masu and stream-type chinook salmon as well as steelhead trout that overwinter in fresh water (Hoar 1976). In nature, fry of these species emerge from the gravel under long spring daylength and then perceive a cycle of long summer days followed by short winter days; lastly, they become smolts in response to increasing daylength in their second year of life. It has been known for some time that exposure of one-summer-old juveniles to long daylength during fall and winter accelerates growth and advances the development of smolt characteristics (Clarke et al. 1978; Zaugg 1981). More recently, it has become apparent that photoperiod conditions during the months immediately following first feeding influence growth and smolting during the first summer (Clarke and Shelbourn 1986). In a hatchery using groundwater or artificially heated water for egg incubation, the time of emergence of fry can be advanced by several months. Under ambient daylight, this has the effect of changing the photoperiod phase at the time of first feeding from long-day to short-day. As a result, growth and smolting may be accelerated (*see* page 537).

*Atlantic Salmon.* Conventional wisdom says that Atlantic salmon parr transform into smolts in the spring under the influence of photoperiod and temperature (*see* Hoar 1988); river discharge together with temperature influence the time of downstream migration (Jonsson and Rudd-Hansen 1985). The smolting process in Atlantic salmon is completed in the spring, having started some months earlier (*see* Kristinsson et al. 1985; Hoar 1988). In production systems using natural temperature regimes, growth slows or stops in the fall when temperature falls to 3-4°C. By that time, any parr that have reached a threshold size and an accumulation of metabolic reserves make the "decision" to commence smolting (Saunders et al. 1994*b*). The consequence of this decision is a surge in growth resulting in these fish distancing themselves from the other parr that have not made this decision. Fish smaller than the threshold size reduce their growth rate under short-day photoperiod in winter (Skilbrei 1991). A visible result is development of bimodality in length frequency, the upper modal fish being on a developmental path for completion of smolting the following spring; the others will not smolt until a year later (Thorpe 1977). The threshold size has been reported to vary between 75 and 100 mm fork length among various stocks of Atlantic salmon (Kristinsson et al. 1985; Skilbrei 1991).

The surge in growth rate resulting in bimodality is the first manifestation of smolting (Kristinsson et al. 1985; Saunders et al. 1994*b*). This is the basis for indicating that smolting starts some months before the final stages of the process ( i.e., silvering, reduced condition factor, development of hypo-osmoregulatory ability, and migratory behaviour) are apparent. Since bimodality develops under a variety of environmental conditions with particular reference to photoperiod and temperature, these environmental factors are probably only permissive. Temperature must be high enough to stimulate feeding and growth. Growth proceeds well under a variety of photoperiod regimes, although a sequence of short and long daylengths is required. Therefore, it appears that there is no specific combination of environmental cues to start the smolting process; it can be initiated under a progression of photoperiod and thermal conditions. Recent work has shown that provision of reasonably high water temperature during the winter allows continued recruitment into the smolting mode (Duston and Saunders 1995*b*). This practice may be used in smolt rearing stations with provisions for elevated temperature in winter.

Temperature and photoperiod serve as important cues for the completion of smolting in the spring and especially for migratory activity. A rising temperature in the 8-10°C range initiates migration of wild smolts (Jonsson and Rudd-Hansen 1985). Increasing daylength in the natural cycle during spring stimulates increases in thyroid hormone activity, hypo-osmoregulatory ability and salinity tolerance (McCormick et al. 1987; Saunders et al. 1989). The two environmental factors work together; increasing daylength alone does not result in development of the smolt characteristics described above unless temperature is suitably high (Virtanen 1988; Duston and Saunders 1995*b*). Similarly, seasonally increasing temperature in the spring may promote growth but with delayed or non-completion of smolting under continuous light conditions (McCormick et al. 1987).

*Rainbow Trout and Charrs.* Within salmonids there is continuum in the developmental stage and the degree to which smolting may be expressed in a given species (Hoar 1988; McCormick 1994). Although species such as brook trout may be generally regarded as non-smolting, they may share some aspects of salinity tolerance with smolting salmonids. Within a species there is a substantial degree of genetic variation in salinity tolerance and smolt characteristics. Such genetic influences are especially problematic when there are conflicting results from different studies on the same species.

## SMOLT PRODUCTION FOR RELEASE

### *Pacific Salmon and Steelhead Trout*

Approximately 75% of the Pacific salmon returning to the Columbia River are derived from hatchery releases. From 1960 to 1990, approximately 40 million yearling coho smolts were released each year from16 hatcheries along the lower Columbia River (Flagg et al. 1995). Annual releases of yearling stream-type chinook salmon from hatcheries in Washington, Oregon and Idaho totalled about 30 million smolts, while annual releases of underyearling ocean-type chinook were in excess of 100 million smolts.

The Alaska Department of Fish and Game increased its production of coho salmon smolts from 171,000 in 1968 to 930,000 by 1976; similarly, its releases of stream-type chinook salmon smolts increased from 400 in 1964 to 225,000 by 1976 (Wahle and Smith 1979). The number of hatcheries rearing chinook salmon in southeast Alaska increased from one in 1971 to 15 in 1992 . Releases are mainly of yearling smolts because releases of underyearlings have not been cost effective; marine survival rates for juveniles released as underyearlings are usually less than 1% whereas survival of yearling smolts averages about 4% (Heard et al. 1995). In addition to traditional land-based hatcheries, biologists in Alaska have developed floating horizontal and vertical raceways for rearing smolts in fresh or brackish water (Martin and Heard 1987).

The central Gulf of Alaska pink salmon hatchery program has developed into a large ocean-ranching system (*see also* Chapter 14). After hatch, the fry are held in seawater netpens for short periods and then released into near-shore nursery areas at a weight of about 0.3 g. Pink salmon were first released from a hatchery on Kodiak Island, Alaska in 1972. By 1990, there were seven private nonprofit pink salmon hatcheries in the central Gulf of Alaska. In Prince William Sound alone, the number of juveniles released rose from 1 million in 1977 to more than 500 million in 1989, exceeding the number of wild migrants in the area.

Alaskan hatcheries incubate about 100 million sockeye salmon eggs per year. Most of the fry produced are stocked into lakes inaccessible to wild salmon and allowed to develop into smolts under natural conditions. Some hatcheries are testing releases of underyearling sockeye smolts at a size of 4-5 g and others are releasing yearling smolts at sizes of 12-20 g.

The Salmonid Enhancement Program was established in 1977 by the Department of Fisheries and Oceans with the aim of restoring catches of Pacific salmon on the west coast of Canada. Many hatcheries were constructed in British Columbia. These used either concrete raceways of the type developed in the Pacific Northwest states (*see* Chapter 6) or earthen channels for rearing of smolts. Hatcheries adjacent to the Strait of Georgia, which separates Vancouver Island from mainland British Columbia, released mainly coho and chinook salmon smolts in support of sport and commercial fisheries. During the 1970s and 1980s, releases of 15- to 25-g yearling coho salmon smolts into the Strait of Georgia increased from about half a million to 7-10 million per year. Marine survival rates ranged from 6 to 20%. Ocean-type chinook smolts were released as underyearlings at a size of 5-7 g; production increased from just over 400 thousand in the early 1970s to 36 million smolts in the late 1980s. Marine survival rates for chinook salmon were highly variable, ranging from 0.3% to 5% (Perry 1995). Hatcheries constructed on the upper Fraser River and its tributaries experimented with release of stream-type chinook salmon as underyearlings at a size of 2.5-5 g but marine survival rates were very low (Cross et al. 1991; Winton and Hilborn 1994). There are no hatcheries producing sockeye salmon smolts in British Columbia; enhancement of sockeye production has been by means of spawning channels and fertilization of sockeye nursery lakes (McDonald and Hume 1984; Hyatt and Stockner 1985; Stockner 1987; West and Mason 1987).

Twenty-two hatcheries now operate on the Pacific coast of Russia, 17 of which are on Sakhalin Island (Dushkina 1994). These hatcheries release mainly pink salmon (450 million) and chum salmon (200 million): the combined production of coho, chinook and sockeye salmon fry totals only 2.8 million.

Hatchery steelhead trout are commonly grown to a size of about 160 mm fork length before release as yearlings. Hatcheries in North America released 30 million steelhead smolts in 1987. Two-thirds of this production was from facilities in the Columbia River basin (Burgner et al. 1992).

### *Atlantic Salmon*

Atlantic salmon pose a special problem in production systems because of their relatively slow growth rate and large smolt size. These factors result in high cost of production. Water temperature and the length of the growing season control growth rate and attainment of the threshold size or energy content to commence smolting (Figure 1). Under culture conditions, it is relatively easy to produce S1 smolts provided water temperature is sufficiently high and the growing season long enough. The optimum temperature for growth is in the 16-18°C range (Siginivich 1967; Peterson and Martin-Robichaud 1989). Failure to provide such temperatures early in the summer and to maintain them for some months results in slower growth than needed to attain the threshold conditions necessary to commence smolting. The result is a low percentage of S1 smolts and the necessity to hold the fish for another year to produce S2 smolts.

Before the development of Atlantic salmon aquaculture in eastern Canada, production from most rearing stations in eastern North America was mainly S2 smolts because the water supplies were mainly from rivers in which water is usually very cold during winter and too warm in summer to promote rapid growth. Some hatcheries had water supplies from stratified lakes which gave an opportunity to regulate temperature in the favourable range; these led the way in S1 smolt production. In Sweden, where the technology for large-scale smolt production was developed during the 1940s and 1950s to compensate for salmon production lost to hydroelectric power development (Carlin 1959), most smolts were S2 because of temperature constraints. Production of S1 smolts in Atlantic Canada and Maine began after the thermal requirements of juvenile salmon were better understood. When the salmon aquaculture industry started in New

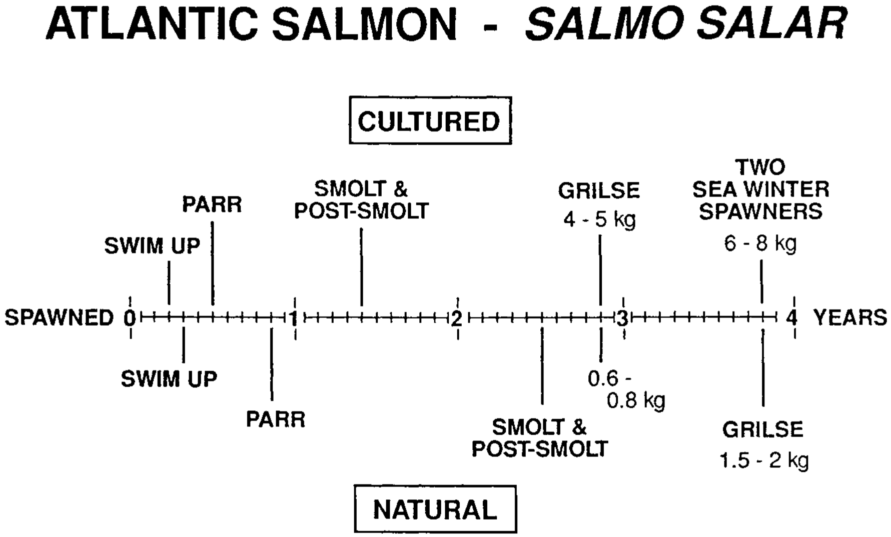

FIGURE 1. Comparison between developmental rates of cultured and naturally produced Atlantic salmon to various life stages. Smolts produced in rearing stations are usually S1 but wild smolts are most commonly S2. Wild smolts in northern rivers are frequently 3-5 years old.

Brunswick in 1978, there were no commercial hatchery-rearing stations, but only government stations for salmon enhancement programs. Smolts supplied to the developing aquaculture industry were a mixture of 1 and 2+, the latter being most common. Now a number of facilities produce smolts for the aquaculture industry. These operators maximize production of S1 smolts to minimize production cost and to optimize use of facilities (Figure 1).

Most smolt production hatcheries use elevated temperature to speed incubation, advance the time of first feeding, and optimize feeding and growth during the summer. For example, the Mactaquac Fish Culture Station of the Canada Department of Fisheries and Oceans uses an accelerated rearing facility with heated water for first feeding and early fry development (Farmer et al. 1990). This approach results in greatly increased S1 smolt production. Cooling water from the bearings at the Mactaquac hydroelectric plant is supplied to a greenhouse facility to which eyed eggs from the Mactaquac Hatchery-Rearing Station are moved about mid-January to begin their accelerated rearing regime. The fry are moved back to the Mactaquac facility in mid-June at a size of about 2 g. Following grading in autumn, about 50% of the fish that are too small to become smolts the next spring are released as fall fingerlings; the remainder are retained as presumptive smolts. By the following April, these fish exceed 60 g and >90% are classified as smolts (G.J. Farmer, pers. comm.).

### *Research on Success of Smolt Releases*

*Effect of Time and Size at Release from Hatcheries on Marine Survival.* Historically, hatchery releases were timed to imitate the downstream migration of wild salmon. Hatcheries also emphasized numbers of fish released. Frequently, more eggs were collected than could be reared to smolt size; the excess were released as fish grew.

Returns of tagged smolts demonstrated that the release period during which maximum return rate can be expected may be as brief as a week (Peterson 1973). In some situations, releases delayed from the normal smolt migration time, either from hatcheries or from netpens near the coast produce a dramatic increase in adult returns (Iioka 1979; Novotny 1980; Eriksson 1991). The increased returns from delayed releases may result at least in part from changes in migratory behaviour that allow salmon to escape fishing mortality or predation (*see also* Chapter 14).

Releases of tagged coho and chinook smolts beginning in the 1960s indicated that larger smolts had better marine survival. For hatcheries in the Pacific Northwest states in 1960, the average release weight for ocean-type chinook salmon was 2.3 g and that for coho and stream-type chinook salmon was 14 g; by 1976, the average weight at the time of release had increased to 7.7 g for ocean-type chinook, 41.2 g for stream-type chinook and 26.7 g for coho salmon (Wahle and Smith 1979). Marine survival of ocean-type chinook salmon was highly correlated with size at release from the Big Qualicum River on Vancouver Island, British Columbia, ranging from 1.9% for 6-g smolts to 9.6% for 12-g smolts (Bilton 1984).

Although general trends in survival could be obtained from analysis of return rates from releases made at different sizes and dates over several years, it was not possible to discern the combined effects of smolt size and release date because of annual variability in the rate of marine survival. This difficulty was overcome by means of experiments examining the simultaneous effects of size and time of release on marine survival. A detailed study of coho salmon involved the release of three size groups on four dates from a research hatchery on the east coast of Vancouver Island, British Columbia (Bilton et al. 1982). Returns of adults and "jacks" (males which become sexually mature in the same year that they enter the sea) to the hatchery and to the commercial and sport fisheries were enumerated. The maximum return of adult coho salmon to the hatchery and to the fisheries was estimated to occur from a release of 25-g smolts on June 22 (Bilton et al. 1982). Releases of larger smolts at earlier dates maximized returns of jacks at the expense of adult returns. Subsequently, similar experiments were performed at a production hatchery on the Quinsam River, Vancouver Island in two years. Analysis of the returns indicated that the effect of release date at this hatchery was much greater than that of smolt size. The maximum returns of adult coho salmon were estimated to occur from a release of 20-30-g smolts on June 5 (Morley et al. 1988). Based on an analysis of several experiments conducted with juveniles released from hatcheries on the Columbia River, Mahnken et al. (1982) recommended that the minimum release size of coho salmon be 130 mm fork length (23 g) in early May or 125 mm fork length (20 g) in early June. They suggested that reversion to parr by fish that were too small at release may be a delayed and unrecognized source of marine mortality in juveniles released from hatcheries.

The effect of size on marine survival of juvenile pink salmon after release was examined at an experimental hatchery on southeast Baranof Island, Alaska (Martin et al. 1981). After hatch, the emerged fry were divided into four groups; one was marked and released unfed into the estuary while the other three were transferred to floating vertical raceways and fed for 30, 60, or 90 days before release. Marine survival of the unfed group (release size 0.23 g) was 3.1%; after feeding for 30 days, mean weight increased to 0.27 g and survival was 4.6% (Martin et al. 1981). Release size increased markedly after 60 and 90 days (0.55 g and 1.95 g, respectively) but survival rates increased only slightly to 5.2% for the 60-day group and then decreased to 4.3% for the 90-day group. When mortality during rearing was taken into consideration, the maximum number of adult returns resulted from the group which was fed for 30 days prior to release (Martin et al. 1981). A similar experiment examined the effect of weight at release on survival of yearling stream-type chinook salmon (Martin and Wertheimer 1989). Differential feeding rates were used to produce large (28-32 g) and small (10 g) smolts after 13.5 months of rearing in floating vertical raceways. Adult return rates (excluding returns of precocious male jacks) were 2.2 - 3% for the large smolts compared with 0.74 - 1.4% for the small smolts (Martin and Wertheimer 1989).

The effect of rearing density in hatchery ponds on smolt quality and rate of adult return is not reviewed in this chapter (*see* Chapters 6 and 7; Fagerlund et al. 1981; Martin and Wertheimer 1989; Banks 1992, 1994; Ewing and Ewing 1995).

*Long-Term Survival Trends in the Pacific.* The introduction of the Oregon Moist Pellet in the early 1960s enabled the release of larger, healthier smolts from hatcheries in the Pacific northwest states. As production of smolts increased markedly during the 1960s and early 1970s, adult returns increased correspondingly. However, returns of adult salmon began to decline noticeably in the late 1970s and early 1980s.

The Little White Salmon National Fish Hatchery was built on the lower Columbia River in 1896 for release of juvenile ocean-type chinook salmon. The hatchery released only unfed fry until 1908 and thereafter increasing numbers were held and fed prior to release at a larger size. Following completion of the Bonneville Dam in 1938, adult returns declined substantially (Nelson and Bodle 1990). Because of low adult returns from 1965 to 1985, the number of eggs transplanted from other rivers exceeded the number of eggs taken from adults returning to the hatchery in 11 out of 21 years (Nelson and

Bodle 1990). Because of low returns and low contribution to the sport and commercial fisheries, the Little White Salmon Hatchery discontinued production of ocean-type chinook salmon. The demise of this stock of chinook salmon cannot be attributed to any single cause, but the construction of the Bonneville Dam and the transplanting of eggs from other stocks are thought to be major factors (Nelson and Bodle 1990).

Coho salmon smolts originating from the Columbia River and coastal streams in Oregon migrate into coastal waters from central California to Washington; this region represents the southernmost production area for coho salmon in the northeast Pacific Ocean. Smolt releases into this area increased from fewer than 1 million in the 1950s to more than 30 million by 1970. However, between 1967 and 1976, catch and escapement of hatchery coho salmon began to fluctuate and then it declined markedly after 1977 (Nickelson 1986). This decline was attributed to a decline in ocean upwelling. Analysis of return rates indicated that survival of smolts released during strong upwelling years was more than twice that of smolts released in poor upwelling years.

These fluctuating ocean conditions affected the survival not just of hatchery smolts but also of wild coho smolts migrating from streams on the west coast of Vancouver Island, British Columbia (Holtby et al. 1990) and of marine populations such as the northern anchovy (*Engraulis mordax*) (Nickelson 1986). Other long-term trends in catches of salmon (including both wild and hatchery-produced) in the north Pacific Ocean have been correlated with changes in the intensity of the Aleutian low pressure system which has a strong influence on oceanographic conditions (Beamish and Bouillon 1993).

Marine survival of coho and chinook smolts produced in British Columbia hatcheries has also varied significantly and tended to decrease since the late 1970s. The average survival rate for coho smolts declined from 14% for 1979 releases to 8% for 1989 releases. For ocean-type chinook salmon over the same period, it ranged from 1.5% down to 0.5%. There was no overall trend for chum salmon; survival of fed fry ranged from 0.6 to 2.4% and for unfed fry from 0.2 to 1.2% for releases between 1979 and 1987. Marine survival of fed pink salmon fry increased from 3.4% in 1980 to 12.4% in 1989; for unfed fry it ranged from 0.7 to 9.9%.

Returns of adult pink salmon to the Prince William Sound area in Alaska increased considerably from the late 1970s to 1990 in parallel with the increase in hatchery releases (Eggers et al. 1996). Marine survival was approximately 3-5%, being higher in years of warmer temperatures during seaward migration than in cooler years.

Virtually all of the Japanese production of chum and pink salmon is supported by hatcheries. The number of returning chum salmon increased from 5 million in the 1960s to 50 million in the 1980s; over the same period, the returns of pink salmon increased from 1.2 million to 3.8 million. These increased returns resulted both from increased hatchery releases and from improved rates of marine survival. Prior to the introduction of manufactured dry diets in the 1965 brood year, marine survival rates for hatcheries in

Hokkaido averaged about 1%; since then they have been 2-3% (Kaeriyama 1996). The marine survival of pink salmon fry released from hatcheries on Sakhalin Island, Russia, is approximately 1-3% and for chum fry, is 2% (Dushkina 1994).

*Atlantic Salmon Programs in Eastern North America and Europe.* Most Atlantic salmon-producing countries adopted the technology developed mainly by Sweden for production and release of smolts in place of the long-practiced release of earlier life stages, i.e., fry and parr (Carlin 1959). Larsson (1980) and Lindroth and Larsson (1985) documented the development of Swedish smolt rearing and release programs and their importance to the Baltic salmon fishery. The Carlin smolt tag provided an effective marking system and allowed meaningful evaluation of smolt releases (Carlin 1955). The tag is small enough to apply to a 10-cm fish. It is retained reasonably well, has an expansion sector to prevent overgrowth during rapid marine growth of the salmon and, therefore, remains visible. Some of its disadvantages were overcome in various modifications by groups using it (Saunders 1968). More recent developments include the magnetic coded wire nose tag (Jefferts et al. 1963) and visual implant tag. The coded wire tag was a major breakthrough because, unlike the external Carlin tag, it does not cause permanent lesions resulting in damage to the fish and increased likelihood of tag loss. The highly visible Carlin tag may attract predators whereas the nose tag is not visible externally. Moreover, the coded wire tag is less expensive and easier to apply. However, both types of tag are still in use, the coded wire tag because it is easy to identify great numbers of fish and the Carlin tag and its modifications where external visibility is important.

The great success with the Swedish smolt release program in the Baltic Sea during the 1950s and 1960s led most salmon-producing countries to attempt similar programs. Tag returns in the Baltic reached 30-40%, largely because Baltic (Atlantic) salmon remain in that sea where there is an intense fishery. Releases of tagged smolts from the west coast of Sweden and from many of the salmon rivers in other parts of Europe and North America gave much lower, highly variable tag returns, owing to more extensive migration patterns, an unexplained higher rate of natural mortality, and exposure to a plethora of distant and local commercial fisheries. In the 1960s, the extensive use of the visible Carlin tag was indispensable in developing knowledge of the growing high seas Atlantic salmon fishery off west Greenland. Saunders et al. (1965) learned that at least some stocks of European and North American salmon feed in those waters during late summer and autumn.

There has been some interest in taking advantage of the homing behaviour of salmon and the smolt production-release technology to develop commercial sea ranching (Thorpe 1980). Most efforts to do this with Atlantic salmon have failed owing to unpredictable and usually low rates of return, and the political problems caused by private versus public ownership and interception in foreign waters. Return rates in some parts of Iceland are high enough to allow commercial sea ranching but this industry is subject to unpredictably low rates of return (*see* Chapter 14).

Smolt-release programs in most countries are aimed at enhancement or re-establishment of wild stocks. Tagged smolts have greatly increased the understanding of salmon migrations and are an important management tool. Declining or lost stocks of salmon have been at least partially restored through smolt release. Much has been learned about the importance of smolt quality, stock origin, and timing and methods of release, which have led to the moderate success of this activity.

## PRODUCTION OF SMOLTS FOR COMMERCIAL CULTURE

### *Coho and Chinook Salmon*

Early commercial salmon farms in Washington State and British Columbia concentrated mainly on coho and to a lesser extent, chinook salmon, which were harvested at a size of 250-350 g (Novotny 1975). Coho and chinook salmon were preferred over pink, chum and sockeye salmon because rearing trials indicated that these latter species were less amenable to netpen rearing because of their greater susceptibility to disease and algal blooms (Brett et al. 1978).

The coho salmon was chosen for its rapid growth, hardiness and availability of surplus eggs from public hatchery facilities. Because most private hatcheries were of limited size and capacity, the managers attempted to make most efficient use of their facilities. This was achieved by producing underyearling smolts. Ground water with a temperature between 7 and 10°C was used to accelerate egg incubation, but at these temperatures many juvenile coho salmon failed to become underyearling smolts in the absence of photoperiod manipulation. Following transfer to seawater netpens at sizes as small as 7 g, these coho salmon frequently became "stunts" and were lost from production (Clarke and Nagahama 1977; Folmar et al. 1982; Nishioka et al. 1982).

As the production strategy shifted to harvest at a larger size, farms in British Columbia began to prefer chinook over coho salmon because its later age at maturity permitted a longer growout period and more flexibility for harvest time. The chinook salmon stocks were mostly of the ocean-type stock, which could be more successfully transferred to seawater at a weight of 7 g. Alternate freshwater rearing methods for chinook salmon were developed to produce larger smolts and thereby reduce the time required for seawater growout. One schedule involved transfer to seawater in October at a weight of 35 g; another involved transfer in March - April at a weight of 50-80 g. These later transfers of larger fish succeeded to shorten the period required for growout in seawater and to bring salmon to market size over a wider season. By 1994, 40% of the chinook smolts transfered to seawater for growout were yearlings. There was also an increase in production time for coho smolts; by 1994, 60% of smolts produced for salmon farms in British Columbia were yearlings.

The annual harvest of coho salmon from Chilean farms is about 30,000 tonnes. Initially, salmon farms in Chile were dependent upon imported eggs. Because of the 6-month phase difference in seasons between the northern and southern hemispheres, commercial smolt suppliers were able to produce 20-30-g coho smolts after nine months without photoperiod manipulation. At present, Chilean farmers produce most of their smolts from their own broodstocks. Elevated temperatures are used in hatcheries to accelerate growth of fry which are then moved to netpens in lakes for rearing to smolt size and transport to the seawater cages as yearlings.

### *Atlantic Salmon*

Farming of Atlantic salmon commenced in Norway in the 1960s and had become a well-established industry by the early 1970s. It grew from 287 farms producing 171 tonnes of Atlantic salmon in 1973 to 791 farms producing 118,000 tonnes of salmon by 1989 (Tilseth et al. 1991). Over this period, the number of commercial smolt producers increased from 17 to 657. Although there has been a consolidation in the industry since 1989, production has continued to expand and about 85 million smolts were transferred to seawater in 1995 (Torrissen et al. 1995). The growth of the Norwegian salmon farming industry was favoured by a long, protected coastline with suitable growing conditions and assisted by government support. Atlantic salmon farming has also developed in Scotland, Ireland, Canada, U.S., Chile and Australia.

The salmon aquaculture industry in eastern Canada started before there was production of smolts specifically for this purpose. Smolts for the first successful commercial venture in New Brunswick were provided from those produced for salmon enhancement and research. Fortuitously, the first smolts used in a commercial venture were from the Saint John River stock (Sutterlin et. al. 1981). In nature these fish usually mature as grilse (after one winter at sea) and as multi-sea-winter salmon in a ratio of about 1:1. Under conditions of cage culture in the southern Bay of Fundy, the incidence of grilse in that first group, harvested in 1979, was insignificant. Subsequently, the incidence of grilse has sometimes been higher but rarely over 10-15%. This low incidence of grilse has been a boon to the local industry. But in other countries and other parts of Canada, the industry has been plagued by early maturity as grilse, a problem since these are smaller and less desirable (and profitable) than multi-sea-winter salmon. Other local stocks of salmon were more likely to mature as grilse and the people in the fledgling industry quickly learned that these should not be used for cage culture.

After the marine aquaculture industry was established in Canada, several private companies began to produce smolts because government facilities could not meet the growing demand. Moreover, the government facilities were mandated to produce smolts for the public, not the private-sector. The private sector smolt producers used a combination of technology already in use by government producers and that transferred from Norway and Scotland where Atlantic salmon aquaculture was already well developed. At first, production of S1 smolts was rarely over 50%, many fish having to be reared a second year as S2 smolts. Modification of the imported technology to take account of the lower water temperature, which restricted growth to incipient smolt size by the end of the first growing season, better husbandry, and improved facilities led to improved S1 smolt production. Meanwhile, research was showing how environmental manipulation and attention to the requirements for smolt development could be used to enhance production of S1 smolts.

## MODIFICATION OF SMOLT AGE AND SEASON

### *Acceleration Using Photoperiod and Temperature*

*Pacific Salmon.* As mentioned earlier, early attempts by salmon farmers to accelerate smolting of coho salmon using elevated temperatures produced variable results. This variability was due to a lack of understanding of the effect of photoperiod (Clarke 1992). Photoperiod exposure during the months after first feeding regulates growth and smolting during the first summer. This was first demonstrated experimentally using photoperiod cycles phased at 1-month intervals (Clarke and Shelbourn 1986). Coho fry were exposed from the time of first feeding to simulated natural photoperiod cycles with phases beginning with either December, January or February daylengths at temperatures of 8, 11 or 14°C. After 6 months of rearing, the coho salmon started on the February photoperiod at 14°C had separated into two distinct size modes. The upper mode had an average weight of 72 g and were able to regulate plasma sodium concentrations to 165 mmol/l after a 24-h seawater challenge. The lower mode had an average weight of 18.9 g and plasma sodium level was elevated to 179 mmol/l after seawater challenge. In contrast, the groups started under December or January daylength had unimodal size distributions and adapted well to seawater. There were corresponding differences in the ability of coho salmon from the three photoperiod phases to grow in seawater; those from the December and January groups grew about five times as fast as did those started under the February photoperiod phase.

The effect of photoperiod phase on smolting of coho salmon is a function of initial daylength. Clarke et. al. (1989) reared coho fry from the time of first feeding under constant 9.5 h (short-day) or 14.5 h (long-day) photoperiod for 2 months and then under simulated natural photoperiod (14.5 h increasing to 17 h) for 4 months. The fry given the initial short-day treatment grew more rapidly and more uniformly in both fresh and salt water than did those given an initial long-day photoperiod. Stream-type chinook salmon respond to photoperiod in the same manner as coho salmon so that the same sequence of short- and long-day photoperiods can be used to produce underyearling smolts (Clarke et al. 1989, 1992, 1994*b*). Masu salmon respond in a similar fashion (Okumoto et al. 1989). It should be noted that this protocol is not effective for Atlantic salmon. The latter species is not responsive to the short-day photoperiod during the first

3 months from start feeding (Clarke 1994). Short-day treatment of juvenile Atlantic salmon is applied only after they have exceeded the threshold size of 7-12 cm fork length (*see* page 539).

Juvenile coho salmon measure daylength not by the accumulated hours of daylight, but by the time during the day when light is experienced. This was demonstrated by Thorarensen and Clarke (1989) using a skeleton photoperiod. Coho fry were divided into nine groups and held under three initial photoperiods (6L:18D, 10L:14D, 14L:10D) for 2 months and subsequently under 3 final photoperiods (16L:8D, 9L:6D:1L:8D, 10L:14D) for three months in a factorial design. Fish exposed first to short-day photoperiods (6L:18D and 10L:14D) and then to a long-day photoperiod (16L:8D) grew faster and had a greater hypo-osmoregulatory capacity in a 24-h seawater challenge test than did any of the other groups. Coho salmon first given short-day photoperiods and then held under the skeleton photoperiod (9L:6D:1L:8D) grew slightly less than those on the complete long-day final photoperiod but substantially better than those kept on the short-day final photoperiod. Coho salmon exposed initially to long-day photoperiod displayed greatly reduced growth and impaired hypo-osmoregulatory capacity in seawater on all subsequent photoperiods, indicating that responsiveness to inductive photoperiods depends on prior photoperiod experience. The effect of the sequence of short-day followed by long-day photoperiods is to change the developmental path taken by underyearling salmon from the parr to the smolt phase of the life cycle. The difference in growth rate between fish on the normal versus accelerated paths is not detectable until about a month after completion of the initial 2-month priming photoperiod.

Light must be excluded completely at night during the period of short-day treatment. Failure to do so will impair growth and smolting. Thorarensen et al. (1989) exposed coho fry to light intensities of 0.0001 - 0.05 lux at night during the initial short-day period. After subsequent exposure to inductive long-day photoperiods, all groups receiving low levels of night illumination had a lower growth rate and poorer hypo-osmoregulatory capacity in a 24-h seawater challenge test than did the control group kept in total darkness at night during the short-day period.

The daylength that must be exceeded to obtain a long-day response is termed the critical daylength (Bünning 1973). For coho salmon fry, the critical daylength during the 2-month priming period is between 11.5 and 12 h (Clarke 1991). Thus, for production of accelerated smolts, the hours of daylight should be kept below this critical daylength for 2 months from first feeding. Ten hours is suggested, but shorter daylengths can be used. Growth of fry will be slightly slower on a 6-h day than on a 10-h day during the priming period.

The effectiveness of a constant short-day photoperiod means that simple light control systems can be used to produce the initial priming photoperiod in a hatchery. At the end of the priming period, the fish can be returned to natural daylength. Short-day conditions can be produced in outdoor tanks by using light-tight covers which are

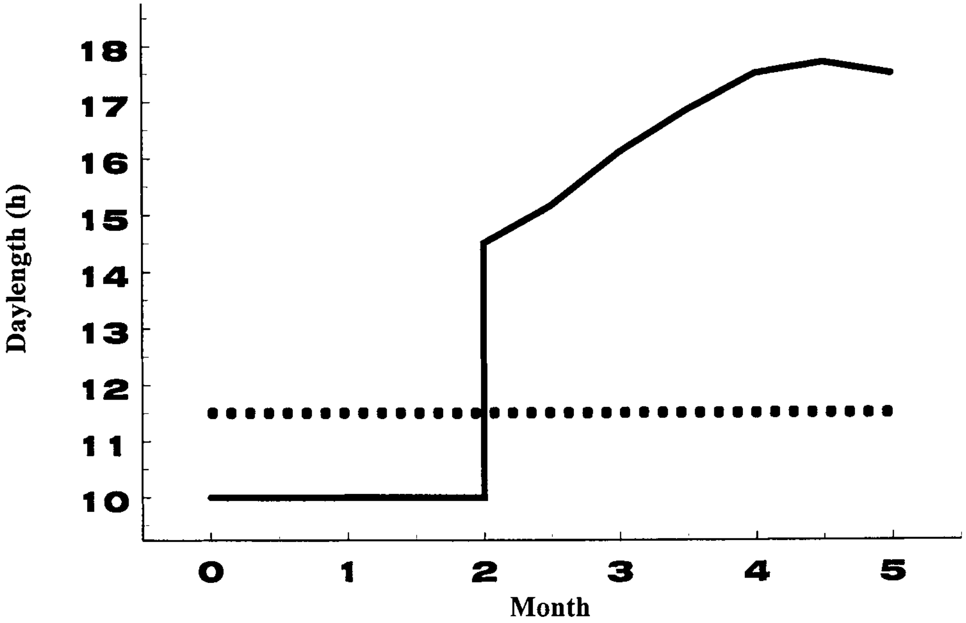

FIGURE 2. A photoperiod schedule for producing underyearling coho salmon smolts. Fry are held under a constant daylength of 10 h during a 2-month priming period in late winter and spring followed by exposure to natural increasing daylength during late spring and summer. The horizontal dotted line indicates the daylength during the priming period below which a short-day response is obtained.

removed and replaced according to a regular schedule each day. Figure 2 illustrates the use of a constant 10-h photoperiod followed by natural increasing spring daylength. Although photoperiod manipulation enables production of underyearling smolts at temperatures as low as 8°C, much larger and more uniform coho salmon smolts are produced at temperatures between 10 and 15°C (Clarke and Shelbourn 1989).

*Atlantic Salmon.* Smolt production, whether for stock enhancement or aquaculture, is best done in the shortest possible time in order to minimize cost and make the most efficient use of facilities. Notwithstanding the usual requirement of two or more years of juvenile growth to reach the smolt stage in nature, artificial production has achieved increasing incidence of S1 smolts by optimizing conditions for rapid growth, notably by maximizing the period with favourable temperature, provision of adequate amounts of high energy feed and practicing good husbandry (Figure 1). Research on the effects of photoperiod on growth and smolting has elucidated the role of daylength controlling the endogenous rhythm of smolting. Moreover, photoperiod affects seasonal patterns of endocrinological activity which in turn, affect growth, development of hypo-osmoregulatory activity and salinity tolerance, and migratory activity (Duston and

Saunders 1990; Saunders et al. 1994*b*). With this knowledge, technology has developed for using photoperiod manipulation to speed growth and thereby increase the number of parr reaching the threshold size or energy level for smolting after one summer's growth (Saunders and Duston 1992). This, together with improved husbandry, has resulted in much improved production of S1 smolts.

A further improvement is production of underyearling (S0) smolts. Research has drawn on the understanding of the role the environmental variables, photoperiod and temperature, to speed juvenile development through the stages leading to smolting. The timetable of development has been advanced wherever possible, i.e., maturation of spawners, incubation of the eggs, development through the alevin stage, first feeding, and fry and parr growth. Spawning is advanced by subjecting the spawners to an artificially advanced regime of decreasing daylength. Incubation is conducted under temperatures higher than those in nature but which have been found compatible with production of reasonably large, viable alevins (Peterson et al. 1977). Similarly, the process of yolk utilization and first feeding is accelerated at elevated temperatures up to 16°C. Early growth through the fry stage is also maximized at temperatures up to 16 °C (Peterson and Martin-Robichaud 1989). Extended daylength from the time of first feeding, which can be as early as January, has been used to speed growth and provide the background for further photoperiod manipulation believed necessary to initiate the smolting process (Saunders et al. 1990). By mid-summer when the parr have reached the threshold size for smolting of about 10 cm, the photoperiod is abruptly reduced to a constant level of about 7 h and held at this level for about 2 months before restoration of a simulated natural or constant long photoperiod (Duston and Saunders 1995*a*). Thereafter, the fish develop smolt characteristics and can be transferred to seawater in November.

Although this system appears to produce viable Atlantic salmon smolts, it is most beneficial in areas where seawater temperatures remain favourable for growth during winter. For salmon farms with low winter temperatures, there is little advantage in putting smolts in seawater that is cooling rapidly to levels at which feeding and growth are minimal. S0 smolts transferred to sea cages in New Brunswick have reached market size somewhat earlier than if they had been reared to smolts and transferred to seawater at the usual times (Duston and Saunders 1995*a*). Continuing research is leading to earlier smolt development and transfer to sea cages to take advantage of favourable temperatures in autumn. A reduction in the time spent in the smolt-rearing station will lead to more efficient use of facilities and lower cost of production. Another objective in production of S0 and other off-season smolts is to achieve a more even annual production of market-sized fish. Duston and Saunders (1995*a*) succeeded in producing underyearling (0+) smolts in 10 months by subjecting fry to a long photoperiod (17L:7D) followed by one or two months of short photoperiod (7L:17D) and a return to long photoperiod. There is convincing evidence that salmonids perceive a long or short photoperiod in relation to previous photoperiod history; days must be longer or shorter than the previous daylength (Randall et al. 1991; Berge et al. 1995; Duston and Saunders

1995*a*; Sigholt et al. 1995). Further research should lead to reducing the seasonal fluctuations in production or directing production to periods of peak market demand.

### *Studies on Performance of Smolts Produced for Commercial Farms*

*Pacific Salmon.* Because growth and survival of salmon held in netpens is more readily observed than that of smolts released to the ocean, it is much easier to determine the reasons for success or failure of a particular batch of smolts. Thus, the development of salmon farming led to a considerable increase in knowledge about smolting and acclimation to seawater. Pioneering studies were conducted by the U.S. National Marine Fisheries Service at a marine experimental facility in Puget Sound, Washington. Survival and growth of groups of underyearling and yearling coho salmon transferred to seawater netpens was monitored for 6 months (Mahnken et al. 1982). Some fish which had the appearance of silvery parr at the time of transfer in April began to regain parr marks after the summer solstice. Unlike the rapidly growing post-smolts, the stunted and parr-revertant coho salmon did not increase in size, so that a bimodal size frequency distribution was apparent by October (Folmar et al. 1982; Mahnken et al. 1982). The size of the largest fish with parr marks at any given time in seawater was found to be a good indicator of the minimum size for survival and growth in seawater; this critical size increased from the summer to winter solstice and then decreased again as the normal smolting time approached in spring. Mortality of the stunts and parr-revertants increased during autumn and winter although a few parr-revertants survived to become smolts the following spring. Similar experiments conducted in France obtained best survival following seawater transfers in September and October for underyearling coho salmon and in April for yearlings; transfers during June and July led to significant mortality due to high water temperatures (Harache et al. 1980; *see also* page 544).

Clarke and Shelbourn (1989) performed a factorial experiment in the laboratory to determine the simultaneous effects of freshwater temperature, seawater temperature, and time of transfer to seawater on growth and hypo-osmoregulatory performance of underyearling coho smolts. A delayed photoperiod was used to ensure synchronous development of underyearling smolts and groups were transferred to seawater at intervals from May 8 to August 28 at mean weights ranging from 4.7 g to 49.6 g. Hypo-osmoregulatory capacity in a 24-h seawater challenge test reached a maximum first at higher freshwater rearing temperatures, ranging from July 5 at 15°C to July 12 at 11°C and July 24 at 8°C. Growth in seawater relative to that in fresh water was fastest when fish were transferred to temperatures higher than their freshwater rearing temperature. The transfer date for maximal growth rate in seawater relative to that in fresh water was not affected by seawater temperatures in the range of 8.9 to 15.8°C. At the end of the experiment in mid-October, the mean weight was greatest for groups reared in 14-15°C fresh water and transferred to seawater in May. Growth was also more uniform in coho salmon reared at higher freshwater temperatures. The coefficient of variation for final weight was much less for coho salmon that had been reared in fresh water at temperatures of 11-15°C compared with those reared at temperatures of 7-8°C (Clarke and Shelbourn 1989).

A similar factorial experiment examined the performance of underyearling ocean-type chinook salmon transferred from fresh water (7-17°C) to seawater (9.5-14.5°C) at intervals from April to June (Clarke and Shelbourn 1985). The optimum hypo-osmoregulatory performance measured in a 24-h seawater challenge test was estimated to occur after transfer of 5.6-g chinook salmon from 13.8°C fresh water to 10.2°C seawater. Maximal growth in seawater relative to that in fresh water occurred with transfer of 6-g fish from 9.7°C fresh water to 14.1°C seawater (Clarke and Shelbourn 1985). Relative growth in seawater declined sharply at temperatures above 14°C. There was considerable mortality during the first month in seawater in groups reared in 16°C fresh water; this was associated with extensive descaling which started in fresh water and persisted after transfer to seawater. This tendency for descaling of larger ocean-type chinook salmon is a concern for hatcheries producing large smolts for offseason transfer. If ocean-type chinook salmon stocks are used, the rearing density must be lowered for fish larger than 10 g. An alternative is to use stream-type chinook or their hybrids, both of which have a much greater resistance to descaling (Clarke et al. 1994*b*).

Betaine has been known as an osmolyte in plants and microbes for some time but is now used to facilitate entry of smolts to seawater. Based on experiments with Atlantic salmon and rainbow trout (*see* below), many commercial salmon farms prepare their smolts for seawater transfer by feeding a betaine/amino acid mixture for 6 weeks before and after the transfer. An experiment conducted with yearling ocean-type chinook salmon confirmed that this dietary supplement produced a significantly greater growth rate and lower plasma sodium concentration after transfer to seawater netpens (Clarke et al. 1994*a*). Individually tagged yearling chinook salmon were fed either a control diet or one containing 1% betaine for 6 weeks in fresh water and an additional 8 weeks following entry into seawater in early April. Hypo-osmoregulatory performance in 24-h seawater challenge tests was not significantly affected by feeding betaine for 5, 47, or 70 days. However, the plasma sodium concentration was significantly lower in the betaine-fed group after 8 weeks in seawater. Growth after transfer to seawater was significantly greater in the group receiving betaine. Examination of individual growth rates revealed that the effect of betaine was to reduce the number of fish growing poorly (i.e., <0.5% body weight · $\text{day}^{-1}$); if these poor performers were removed from the analysis, there was no longer a significant effect of betaine on growth rate.

*Atlantic Salmon.* Based on experience with transferring of New Brunswick stocks of smolts to sea cages in the Bay of Fundy, temporal and thermal constraints strongly affect survival and growth performance. The spring-time "window" for smolt transfer in the Fundy area is from approximately mid-April to late May. It became apparent during the early development of the industry that smolts should not experience freshwater temperatures above 12°C in the spring before transfer. Temperatures of 13-16°C result in a loss of gill $Na^+,K^+$-ATPase activity and salinity tolerance. Because surface water supplies to smolt rearing stations warm quickly in May, there is a very narrow window for successful smolt transfer; this period was the month of May in the early years of the industry, and in some cases only the first half of May for rearing stations with warmer water. This necessitated moving smolts in late April when sea temperature is as low as 5°C. This was found not to be a constraint and many producers were forced to transfer many of their smolts in April when both fresh and seawater temperatures were 5°C or lower. The consequences of such early transfer were usually favourable, in contrast with those done later after freshwater temperatures had risen to 15°C or higher. Seawater temperatures in the Fundy region rise from about 5°C in mid April to 8-10°C in late June.

Smolt size is of great importance for initial survival after transfer to seawater and for long-term growth performance. Although smaller smolts sometimes survive and grow well, it is preferable to stock out smolts of 16 cm or larger. Smaller individuals are likely to be non-smolts or previously mature males, both of which may not survive and grow well. Some smolt producers mistakenly hold their fish in fresh water as long as possible in the spring, hoping to have them reach smolt size before the latest acceptable time of transfer to seawater. Such fish have not made the earlier "decision" to become smolts and will not do so that season in spite of having reached smolt size in the spring. For the Saint John River stock used extensively in the Bay of Fundy, large smolts (>20 cm) are more likely to mature as grilse than are smaller individuals. Therefore, large S2 smolts and similarly large S1s are more likely to mature as grilse than their smaller (<18 cm) siblings. For the Saint John stock reared in the Bay of Fundy, the optimum smolt is an S1 from 18 to 20 cm because large smolt size is positively correlated with large size at harvest (O'Flynn et al. 1995).

The Norwegian Research Council initiated a program to improve smolt quality in their salmon farming industry in 1990. Staff of companies producing smolts for sale to salmon farms have been trained in the determination of smolt quality (*see* page 545). During the past few years, this increased focus on smolt quality has helped Norwegian salmon farms to reduce considerably the mortality of smolts after transfer to seawater netpens. Another component of the program developed photoperiod treatments for production of underyearling smolts (*see* page 540). Minimizing handling stress, for example by using water-to-water transfers, can greatly reduce mortality associated with entry to seawater (Flagg and Harrell 1990).

Supplementation of the diet with a betaine/amino acid mixuture before and after transfer to seawater can facilitate hypo-osmoregulation by Atlantic salmon smolts, as discussed earlier for Pacific salmon. Yearling Atlantic salmon fed 1.5% betaine in the diet for 2 months in fresh water had an improved hypo-osmoregulatory performance in a 48-h seawater challenge at a salinity of 39‰, elevated gill $Na^+,K^+$-ATPase, and an elevated plasma thyroxine concentration compared with those fed a control diet (Virtanen et al. 1989). Subsequent studies with different levels of betaine supplementation indicated that 1% betaine supplementation in smolt diets, starting 6-8 weeks before transfer to seawater and for 3 weeks afterward will reduce weight loss and hasten the recovery of appetite (Virtanen et al. 1994). However, Duston (1993) reported that feeding Atlantic salmon parr in fresh water for 25 days with 1.5% betaine did not influence growth, gill $Na^+,K^+$ ATPase activity, or plasma osmolality after transfer to seawater during autumn.

### *Offseason Transfers*

The highly seasonal nature of the smolting process under natural conditions can be an impediment to salmon farming. One problem for salmon farms is the seasonal fluctuation in salmon harvest associated with the normal life cycle. It is desirable to spread harvests across seasons to minimize seasonal variation and provide a more constant amount of salmon for market throughout the year. In some regions, unfavourable conditions in the ocean due to seasonal temperature extremes or algae blooms need to be avoided. Therefore, techniques for offseason transfers of smolts have been developed to meet the requirements of industry.

*Coho Salmon.* Harache et al. (1980) demonstrated that one-summer-old (S0) coho salmon could be transferred successfully to 35‰ seawater in Brittany, France, after temperatures dropped below 16-17°C. The fish had to be larger than 170 mm fork length by September or 210 mm by late December in order to avoid growth stunting. Japanese salmon farms transfer underyearling coho salmon to seawater netpens at a minimum size of 120 g from mid October to early December when seawater temperatures drop below 17-18°C (Iwata and Clarke 1987).

*Atlantic Salmon.* Considering the progress made in reducing the time to produce smolts and success with transferring them to seawater at off-season times, it is likely that smolts can eventually be produced throughout the year by using environmental manipulation. The practicality of doing so will depend largely on local environmental conditions. In regions with little variation in sea temperature during the year there may be greater practicality in off-season smolt production and transfer to seawater than in regions with wider extremes in sea temperature. Frantsi and Justason (1988) noted that off-season transfer of yearling Atlantic salmon could be used to increase hatchery efficiency and expand the harvest season in New Brunswick salmon farms. Research in Norway has been directed at autumn transfer of underyearling Atlantic salmon. This practice has developed considerably in recent years and by 1995, 25% of all smolts transferred to netpens on Norwegian salmon farms were underyearlings transferred in autumn (T. Hansen, Matre Aquaculture Station, Matredal Norway, pers. comm.).

## TECHNIQUES FOR ASSESSING SMOLT READINESS AND QUALITY

### *Salinity Tolerance Test*

One of the prime requirements of a smolt is that it must be able to tolerate abrupt transfer from fresh water to seawater and continue growing there. A simple test has been developed in which presumptive smolts are transferred directly from fresh water to hypersaline water (salinity 37-40‰) and observed for 96 h (Adams et al. 1975; Komourdjian et al. 1976; Saunders et al. 1985). Those surviving for this time are capable of direct transfer to normal salinities (30-32‰). It has usually been observed that salmon with such high salinity tolerance also have elevated gill $Na^+,K^+$ ATPase activity. The salinity tolerance test is an easy and a reliable way of determining whether or not a given lot of smolts is ready for transfer to seawater. Hypersaline water must be used for the test because tolerance of 30‰ seawater is not an adequate criterion to identify smolts; large parr may survive indefinitely but fail to grow adequately (Clarke and Nagahama 1977; Harache et al. 1980; Folmar et al. 1982).

The salinity tolerance test is conducted in temperature-controlled tanks (usually 100-200 l) filled with water made up to a salinity of 37.5‰ by mixing appropriate amounts of a salt mixture (Instant Ocean® or measured amounts of the principal salts in natural seawater). Temperature is usually maintained at the same level as that to which the fish have been acclimated in their rearing tanks. Temperatures higher or lower than those during recent experience may be used to learn how smolts may respond after transfer to sea cages where temperature may be far different from that recently experienced by the smolts. The fish are fasted for 24 h prior to being transferred from their freshwater holding tanks directly to the salinity baths. Feed is not offered during the 96-h test. Dead fish, if any, are removed daily. The results are expressed as percent survival after 96 h.

Johnsson and Clarke (1988) suggested that the period of the test be extended to 144 h from 96 h at low temperatures and when the fish are larger than 50 g in order to increase the test's sensitivity. In order to determine the seasonal development of salinity tolerance and analyze the results statistically, it is possible to perform a series of tolerance tests at different salinities. As mortality rises quickly with increasing salinity, it is advisable to use five salinities in increments of 1 or 2‰ in order to obtain at least two or three concentrations giving mortalities in the range between 2 and 98% (Blackburn and Clarke 1987; Johnsson and Clarke 1988). This enables a calculation of the 96-h median lethal salinity with confidence limits by means of probit analysis. The 96-h LC50 test has been used with juvenile rainbow and steelhead trout (Johnsson and Clarke 1988), chinook, coho and chum salmon (Clarke et al. 1989) and pink salmon (Varnavsky et al. 1991). Table 1 summarizes LC50 salinities measured for various species as they grow.

The 96-h LC50 test can use 100 juveniles (20 fish at each of five salinities) or more. When limited numbers of larger fish are available, the 24-h seawater challenge

TABLE 1.

The 96-h LC50 salinity measured for various species in relation to size.

| Species | Weight or fork length | Temperature °C | LC50 salinity | Source |
|---|---|---|---|---|
| Rainbow trout | 9.5 g | 11 | 29.4 | 1 |
| | 33.0 g | 11 | 35.1 | 1 |
| | 95.0 g | 11 | 35.8 | 1 |
| Steelhead trout | 4.0 g | 11 | 28.9 | 1 |
| | 10.4 g | 11 | 31.4 | 1 |
| | 24.6 g | 11 | 35.1 | 1 |
| | 46.9 g | 11 | 37.4 | 1 |
| | 80.6 g | 11 | 39.4 | 1 |
| Chum salmon | 42 mm | 10 | 42 | 2 |
| | 54 mm | 10 | 46 | 2 |
| | 71.2 mm | 10 | 50.0[1] | 2 |
| Coho salmon [2] | 34.5 mm | 10 | 26.5 | 2 |
| | 46.1 mm | 10 | 32.1 | 2 |
| | 53.1 mm | 10 | 34.2 | 2 |
| | 62.7 mm | 10 | 35.4 | 2 |
| | 95.7 mm | 10 | 40.8 | 2 |
| Chinook salmon [3] | 37.6 mm | 10 | 25 | 2 |
| | 45.0 mm | 10 | 28.2 | 2 |
| | 56.4 mm | 10 | 34.5 | 2 |
| | 70.4 mm | 10 | 36.2 | 2 |
| | 100.7 mm | 10 | 41.4 | 2 |
| Pink salmon | 0.2 g | 7 | 39.2- 40.4 | 3 |
| | 0.5 g | 7 | 38.9 - 39.2 | 3 |

Sources: 1-Johnsson and Clarke (1988); 2-Clarke et al. (1989); 3-Varnavsky et al. (1991).

[1] Extrapolated value

[2] Accelerated photoperiod applied to produce underyearling smolts

[3] Ocean-type stock

plasma sodium test can be used as an alternative (*see* below). The latter test is also has the benefit that it does not usually cause death of the fish. In view of increasing regard for animal welfare, the use of the salinity tolerance test rather than alternate procedures that cause less distress and mortality may have to be justified.

### *Seawater Challenge Hypo-osmoregulatory Test*

The seawater challenge hypo-osmoregulatory test measures the ability of salmonids to regulate plasma ion concentrations shortly after their abrupt transfer to seawater. The seawater challenge test was developed initially to measure the readiness of accelerated underyearling coho salmon for transfer to seawater netpens for growout (Clarke and Blackburn 1977). This test was chosen as an indicator because it demonstrated a regular developmental pattern even at the elevated temperatures used for growth acceleration, unlike other indicators such as lipid content and condition factor (Clarke et al. 1978; Clarke 1982). Furthermore, this test is simpler and cheaper to perform than assays for gill $Na^+,K^+$-ATPase or plasma thyroxine. However, for species such as chum and pink salmon that may be too small from which to collect a blood sample, the salinity tolerance test can be used as an alternative.

The seawater challenge test involves transfer of a sample of fish to seawater at their acclimation temperature; after 24 h, the fish are anesthetized and a blood sample is collected. Plasma is separated from the blood cells by centrifugation and then diluted for measurement of sodium concentration using a flame photometer, atomic absorption spectrophotometer or sodium ion analyzer (Blackburn and Clarke 1987). Alternatively, osmolality can be measured in undiluted plasma using an osmometer (Grau et al. 1985); this practice has been adopted in hatcheries operated by the Department of Fisheries and Oceans in British Columbia. A third option is to measure plasma chloride concentration using a chloride titrator (Sigholt et al. 1989). Many commercial hatcheries in Norway and Canada now use plasma chloride concentration as an indicator of performance in the seawater challenge test, the advantage being that a chloridometer is relatively inexpensive and easy to use. Expected plasma sodium concentration in smolts after a 24-h challenge is approximately 160-165 mmol/l. For plasma osmolality, the corresponding value is 330-340 mosmol/l and for plasma chloride 140-150 mmol/l. Mortality in the test indicates very poor hypo-osmoregulatory capacity and may be indicative of gill damage or significant health problems in the smolts.

The 24-h interval for the challenge test was chosen by Blackburn and Clarke (1987) for coho salmon at 10°C because plasma sodium concentrations were maximal at that time. However, the maximum may occur earlier or later than this in relation to temperature and species used. The maximum rise in levels of sodium ion in juvenile chum (Iwata et al. 1982) and pink salmon (Varnavsky et al. 1991) occurred after 12 h in seawater. Virtanen and Oikari (1984) reported that plasma sodium was higher at 48 h than at 24 h after transfer of juvenile Atlantic salmon to seawater at 1.5°C.

Clarke and Blackburn (1977) used a salinity of 30‰ for the challenge test because it was readily available. More recently, a salinity of 35‰ has been produced by adding salts to natural seawater (Berge et al. 1995; Sigholt et al. 1995). Although more time and effort is required to make this higher salinity, its use improves the ability of the test to detect smolts with a fully developed ability to osmoregulate in seawater, particularly when the fish are larger than 80 g. The use of 35‰ seawater is now a common practice for testing of Atlantic salmon smolts produced in commerical hatcheries in Norway.

A balanced salt mixture *must* be used to make artificial seawater because single ion solutions are toxic (Hoar 1966; Blackburn and Clarke 1987). Addition of a precise weight of salt might not produce the calculated salinity because of variable amounts of moisture absorbed during storage. Therefore, it is advisable to measure the salinity after dissolving the salt and make adjustments as necessary to achieve the intended concentration. It is important that salinity be accurate to within 1‰ for results to be comparable among tests. Salinity can be measured using a hydrometer, refractometer or conductivity meter; if a chloride titrator is used, salinity can also be calculated from the chloride concentration according to the following equation:

$$\text{salinity (‰)} = 0.03 + [1.805 \cdot \text{chloride (mmol/l)} \cdot 0.035453] \qquad (1)$$

The salt should be added to the water at least a day before the test to ensure that it is dissolved thoroughly. Spurious mortality may be observed when using freshly made artificial seawater. Fewer toxicity problems are observed when salt is added to ordinary seawater to bring the salinity up to the desired concentration.

A single test will not indicate whether hypo-osmoregulatory capacity is increasing or decreasing. Therefore, it is worthwhile to perform the test at regular intervals during the last 2 to 3 months before seawater entry. This allows the development of the smolts to be examined so that the optimum time for seawater entry can be estimated well in advance. Other factors that can influence the results obtained from the seawater challenge test include seawater temperature, density of the fish in the test tank, contamination of the blood samples with seawater, and descaling of the smolts. Temperature in the test tanks should be close to the acclimation temperature for the smolts. The risk of contamination can be minimized by rinsing the smolts in fresh water and blotting them dry before taking a blood sample.

In addition to Pacific and Atlantic salmon, the seawater challenge test has been used for rainbow and steelhead trout, sea (brown) trout, Arctic charr, Dolly Varden charr (*Salvelinus malma*) and coastal cutthroat trout (*Oncorhynchus clarki*) (Hogstrand and Haux 1985; Blackburn and Clarke 1987; Johnson and Heifetz 1988; Yeoh et al. 1991; Arnesen et al. 1992; Johnsson et al. 1994).

### *Gill $Na^+,K^+$-ATPase Activity*

Gill $Na^+,K^+$-ATPase is an ion-translocating enzyme involved in salt secretion of fish in seawater (Silva et al. 1977). In smolts there is generally a strong correlation between seasonal increases in salinity tolerance and gill $Na^+,K^+$-ATPase activity, and this enzyme has been widely used as a 'marker' for the parr-smolt transformation (McCormick and Saunders 1987; Hoar 1988). There are several useful techniques for measurement of gill $Na^+,K^+$-ATPase activity of teleosts (Johnson et al. 1977; Zaugg 1982; Mayer-Gostan and Lemaire 1991), including a "microassay" suitable for non-lethal biopsies and organ culture (McCormick 1993; Schrock et al. 1994). Measurement of $Na^+,K^+$-ATPase is most frequently accomplished by measurement of $V_{max}$ in a microsomal preparation or crude homogenate and expressed as activity per unit wet weight of protein. A higher specific activity is obtained when using microsomal preparations compared with crude homogenates, but smolt-related increases are comparable with both methods. The breakdown of adenosine triphosphate (ATP) is detected (most often by measuring inorganic phosphate or adenosine diphosphate, ADP) in the presence and absence of ouabain, a specific inhibitor of $Na^+,K^+$-ATPase. Such biochemical measurements are, by their nature, method-dependent, and efforts should be made to validate and optimize assay conditions for expression of enzyme activity, including tissue preparation and amount, ion concentrations, pH, ouabain, detergent and temperature. As with salinity challenge tests, a time series is necessary to determine smolt status accurately.

### *Hormone Assays*

Measurement of hormones involved in smolting can be useful for following smolt development and effects of husbandry processes on growth and salinity tolerance. Thyroid hormones, cortisol, growth hormone, insulin-like growth factor I and prolactin have all been implicated in regulating the osmoregulatory changes that occur during smolting (Hoar 1988; McCormick 1994). These hormones, pancreatic peptides and others may also be involved in growth and metabolic changes during smolting (Hoar 1988). Radioimmunoassays applicable to measurement of hormones in salmonid plasma have been developed for thyroid hormones (Dickhoff et al. 1978), cortisol (Redding et al. 1984), growth hormone (Bolton et al. 1986; Le Bail et al. 1991), insulin-like growth factor I (Moriyama et al. 1994) and prolactin (Hirano et al. 1985). It should be noted that these assays can be relatively expensive and require both technical and interpretive expertise. Non-radioactive enzyme immunoassays (e.g., enzyme-linked immunosorbant assay or ELISA) have been developed for measurement of plasma cortisol in salmonids (Barry et al. 1993; McCormick, unpubl. data). Although commercial assays for thyroid hormones and cortisol developed for use in mammals may be applied to fish, their validity requires careful evaluation.

### *Other Tests: Weight Changes, Enzymes, Silvering*

*Silvering.* Although smolts are usually silvery in colour, this criterion is not universally reliable as an indicator of smolt status in reared Atlantic or Pacific salmon (Wedemeyer et al. 1980). Feed components and perhaps tank colour can result in parr becoming silvery. Such fish, particularly those reared under inappropriate photoperiod or temperature regimes often fail to develop or do not maintain salinity tolerance or elevated gill $Na^+,K^+$-ATPase activity in spite of having large size (McCormick et al. 1987; Duston et al. 1991). Duston (1995) has developed a light reflectance meter to measure silvering of smolts.

*Metabolic Enzymes.* The concentration of liver mitochondria and the activities of the respiratory chain enzyme, cytochrome c oxidase and another mitochondrial enzyme, citrate synthase, are higher in smolts than in parr (Blake et al. 1984; McCormick et. al. 1989).

*Condition Factor and Morphology.* The smolting process is characterized by changes in the dynamics of growth. This is often reflected in the relation between length and weight. Condition factor provides a measure of weight per unit length. It is calculated as [weight·100·length$^{-3}$] where weight is measured in grams and fork length is measured in centimetres (*see also* Chapter 2). Smolts reared under natural conditions have a lower condition factor than parr due to faster growth in length (particularly in the tail region) than in weight (Winans and Nishioka 1987; Beeman et al. 1994). Condition factor varies among species and is influenced by temperature and feeding rate. Juveniles which are accelerated by rearing at high temperature and on a high energy ration may exhibit little or no decline in condition factor during smolting. For this reason, condition factor is perhaps best used as a simple measure of body shape of fish produced in a particular hatchery over time. Variation in condition factor among groups of smolts produced at hatcheries with distinct rearing environments are not readily interpreted as differences in smolt quality.

More detailed morphometric measurements can be made using a digitizing board attached to a computer. A large number of morphological traits can be examined using principal components analysis. Winans and Nishioka (1987) first used this procedure to describe changes in body shape of hatchery-reared coho salmon during smolting. The technique has also been applied to stream-type chinook salmon and steelhead trout smolts (Beeman et al. 1994, 1995). An advantage of this method is that it is suited to assessment of smolts when lethal sampling is not possible. The morphometric measurements can be taken from photographs of live smolts. However, it does require a larger sample size than do the more invasive methods.

*Weight Change in Seawater.* Small but measurable changes in length and weight of juvenile salmonids occur due to dehydration after transfer to seawater. Zaugg and Beckman (1990) found that these decreases in length and weight of juvenile coho salmon transferred to 31‰ seawater for 24 h were less pronounced during the period when the level of gill $Na^+,K^+$-ATPase was greatest. They suggested that this phenomenon could be used as a simple test of seawater readiness. Blackburn and Clarke (1987) measured weight loss in juvenile coho salmon, steelhead trout and domesticated rainbow trout in 24-h seawater challenge tests. In five out of six groups, the correlation between weight loss and plasma sodium concentrations was significant. However, the slopes and intercepts were not homogeneous, indicating that the relationship would have to be calibrated separately for each stock being tested. The coefficient of variation for weight loss is much larger than for the seawater challenge blood sodium test, so that larger sample sizes are required to detect differences among groups. Varnavsky et al. (1991) reported that weight loss in juvenile pink salmon was maximal 12 h after transfer to 30‰ seawater.

*Cataracts*. The formation of ocular cataracts (opacity of the lens) due to osmotic stress has been studied in coho salmon (Iwata et al. 1987). In a series of transfers of yearling coho salmon to seawater at different seasons, increased cataract formation was associated with a decreased ability to acclimate to seawater. Iwata et al. (1987) proposed that the occurrence of cataracts after transfer to seawater may provide a simple method for assessing smolt status. However, this test has not gained wide acceptance.

## TRANSFER OF NON-SMOLTING SPECIES TO SALT WATER

### *Rainbow Trout*

As in most salmonids, larger rainbow trout have increased survival following seawater exposure. Jackson (1981) found that mortality and plasma osmolality decreased with increasing size for fish between 11 and 30 g following transfer to 30‰. Rainbow trout smaller than 15 g can survive direct transfer to salinities <22‰ (Landless 1976). Since osmotic perturbations can increase with relatively small increases in salinity and changes in temperature (*see* below), the size necessary for maximum seawater survival will depend on environmental conditions. Weight > 50 g would appear to be a minimum for direct transfers to salinities > 30‰ (Landless 1976; Jackson 1981).

The salinity of coastal seawater can vary greatly and is an important consideration for growth and survival of rainbow trout. Johnsson and Clarke (1988) found that 7-15 g rainbow trout grew equally well at 14 and 19‰ but growth was reduced at 24‰. For 30-g fish mortality and osmotic perturbations increase with salinities greater than 15‰, and the rate of increase in plasma osmolality increases more rapidly and to higher levels in 32‰ than in 28‰ (Jackson 1981). Jürss et al. (1986) reported that for 40 to 60-g fish growth rate was lower for fish at 20‰ than at 8‰ or in fresh water. For 51- to 153-g rainbow trout, growth rate decreased with increasing salinity (20-32‰) relative to fish in fresh water and 10‰ (McKay and Gjerde 1985). MacLeod (1977) found that food intake of 40-g rainbow trout was significantly lower at 32.5‰ than at lower salinities (0, 7.5, 15 and 28‰) and that absorption efficiency decreased with increasing salinity. This study also demonstrated that increases in salinity of 7.5-13‰ resulted in transitory decreases in food intake and growth rate that recovered within 14 days. The findings of the latter study underscore a weakness present in many of the above studies, which are generally short term and may be unable to distinguish an initial decrease in growth caused by salinity acclimation as opposed to long-term growth following acclimation.

Although we know of no published studies with rainbow trout, work with other salmonids suggests that gradual acclimation will increase survival (Boeuf and Harache 1984). In support of this idea, Johnston and Cheverie (1985) reported improved survival, growth and ionoregulation in rainbow trout in an estuary with fluctuating salinity (17-29‰) than one with constant, high salinity (28-29‰). Inclusion of salt in the diet for a period before transfer can also improve the salinity tolerance of small (<40 g) rainbow trout to 36‰ seawater (Salman and Eddy 1990).

Temperature is an important factor in survival and growth of rainbow trout in seawater. Saunders (1975) reported that rainbow trout previously acclimated to 30‰ salinity and that subsequently experienced seasonal decreases in temperature suffered high mortality when temperatures dropped below 0°C. Rainbow trout previously reared in 8°C fresh water experienced much higher mortality, and osmotic and ionic disequilibrium when transferred to 1°C seawater (26‰) than 8°C seawater (Sigholt and Finstad 1990). Johnsson and Clarke (1988) reported that in isothermal transfers of 10- to 90-g fish, salinity tolerance (as judged by a 96-h LC50 test) was greatest at 11°C, lowest at 17°C and intermediate at 5°C. It will be important to consider not only the absolute temperature at the time of transfer, but also the temperature difference from the freshwater rearing conditions. Jürss et al. (1987) found that in 55-g rainbow trout in 20‰, growth was greatest at 11°C, followed in order by that at 6°C, 16°C and 1°C. These studies suggest that the optimal temperatures for seawater growth of rainbow trout are in the 8-14°C range.

Only limited information exists on seasonal changes in salinity tolerance and seawater growth of rainbow trout. The effects of temperature discussed above suggest that in conditions of seasonally changing seawater temperatures, salinity tolerance would be lowest in winter and summer. Johnsson and Clarke (1988) found no effect of photoperiod on salinity tolerance of rainbow trout, whereas increased daylength increased salinity tolerance in steelhead trout. However in another study, spring increases in gill $Na^+,K^+$-ATPase activity were found in domesticated rainbow trout held at relatively constant temperature (10 - 13°C), suggesting the possibility of temperature-independent seasonal increases in salinity tolerance (Rey et al. 1990). Smith and Thorpe (1976) reported that in spring at high ration levels, rainbow trout had a higher growth rate and nitrogen assimilation efficiency in seawater relative to fish in fresh water; this difference was not detected in autumn even though the tests were conducted at the same temperature. Hybridization with domesticated rainbow trout reduced the seasonal variation in freshwater growth of anadromous steelhead trout; the seasonal decline in growth rate during winter was more pronounced in steelhead than in half-sib rainbow-steelhead hybrids or in domesticated rainbow trout (Johnsson et al. 1993). Moreover, the hybrid trout had a greater hypo-osmoregulatory capacity than did steelhead trout as shown in 24-h seawater challenge tests during winter whereas the steelhead had a greater hypo-osmoregulatory capacity during the normal smolting season in May (Johnsson et al. 1994).

### *Brown Trout*

Not nearly as much is known of the ontogeny and environmental influences on salinity tolerance of brown trout compared to present knowledge of rainbow trout. Salinity tolerance increases with increasing size (Parry 1958; Boeuf and Harache 1982; Tanguy et al. 1994), although information on a clear size threshold for seawater transfer is lacking and will likely depend on the stock and environmental conditions. Gordon (1959) found that salinity tolerance was lower in summer than from September through April and suggested this was due to high summer temperatures. Boeuf and Harache (1982) found smolt-like physiological changes in an anadromous stock of brown trout (sea trout) and none in a non-anadromous stock of brown trout, but there were no differences in short-term salinity tolerance between the two forms. Tanguy et al. (1994) have found, however, that sea trout had higher long-term survival in seawater than did brown trout.

### *Arctic Charr*

Size, season, and temperature seem to affect salinity tolerance of Arctic charr in a manner similar to that in rainbow trout. Several reports indicate increasing salinity tolerance with increased size of Arctic charr (Arnesen et al. 1992; Delabbio et al. 1990; Staurnes et al. 1992; Dempson 1993). Size thresholds of 60 and 200 g for introduction to seawater were reported by Staurnes et al. (1992) and Delabbio et al. (1990), respectively; differences in criteria (short- and long-term, respectively) and environmental conditions may account for these discrepancies. Those authors also reported substantially greater salinity tolerance in anadromous Arctic charr relative to non-anadromous strains. Anadromous stocks of Arctic charr spend only short periods of the summer in seawater and none are known to overwinter in the sea (Dempson and Kristofferson 1987).

As with other salmonids, increasing salinity results in greater osmotic perturbations and lower survival in Arctic charr (Arnesen et al. 1993; Dempson 1993). Food intake decreases with increasing salinity for the first several days after exposure, but this difference is no longer detectable after 30 days and has no effect on long-term growth rates (Arnesen et al. 1993). Dempson (1993) suggested that gradual acclimation may be particularly important for fish between 12 and 15 cm.

Seasonal changes reported by several authors indicate that salinity tolerance is greatest in spring (even when temperature remains constant), and that this seasonality is found in landlocked populations (Finstad et al. 1989*a*; Arnesen et al. 1992; Schmitz 1992). In Arctic charr previously acclimated to seawater, plasma ions and mortality increase in winter (Wandsvik and Jobling 1982; Staurnes 1993). This may be primarily a temperature effect, as salinity tolerance is much lower at 1°C than at 8°C (Finstad et al. 1989*a*, 1989*b*), though a temperature-independent seasonality is also possible. Transfer of mature male Arctic charr to seawater results in greater mortality and elevations of plasma sodium relative to immature fish (Staurnes et al. 1994).

### *Brook Trout (Charr)*

McCormick and Naiman (1984*a*) found a strong size-dependence of salinity tolerance of brook trout gradually acclimated to 32‰, with 100% survival of immature fish greater than 17 cm. Although gradual acclimation increases survival in seawater (Pelletier and Besner 1992), direct transfer of 19-21-cm brook trout to 25‰ resulted in 85% survival (Besner and Pelletier 1991). No seasonality in salinity tolerance or gill $Na^+,K^+$-ATPase activity was found in brook trout reared and tested at constant temperature (McCormick and Naiman 1984*a*, 1984*b*). Fish reared under seasonal changes in freshwater temperature and exposed to seasonal changes in seawater temperatures had higher salinity tolerance and gill $Na^+,K^+$-ATPase activity in May than in late summer (Besner and Pelletier 1991). Salinity tolerance of mature male brook trout is substantially less than that of mature females and immature fish, especially during the spawning season (McCormick and Naiman 1985).

### *Dolly Varden and Lake Trout*

Very little information exists on the salinity tolerance of these charr species. Using the 24-h seawater challenge, Johnson and Heifetz (1988) found that wild, migrating Dolly Varden charr had similar hypo-osmoregulatory capacity as migrating coho salmon smolts. Over a relatively narrow size range (11-15 cm), there was no effect of size on salinity tolerance in this species. No published reports on the salinity tolerance of lake trout (*Salvelinus namaycush*) appear to exist. As anadromous populations of lake trout are rare, it is likely that their salinity tolerance is the lowest among the species discussed here.

## AREAS OF CURRENT RESEARCH AND FUTURE RESEARCH NEEDS

One current area of research is the refinement of techniques for producing underyearling and other off-season smolts. This requires additional fundamental research to learn how photoperiod affects the growth processes leading up to the commencement of smolting. Also, the timing and duration of photoperiod manipulation to optimize production needs refinement. Further research is required to produce underyearling smolts earlier in the autumn while sea temperatures are sufficiently high to promote growth in regions with low winter temperature.

Research is continuing to increase the predictability of performance after transfer of smolts to seawater netpens, particularly to shorten the interval during which food consumption is reduced (Usher et al. 1991; Stradmeyer 1994). Knowledge of the genetic control of smolting is limited for most species. Considerable potential remains for selective breeding to improve smolt yield under intensive culture, particularly as underyearlings (Withler et al. 1995).

Another area offering great promise in future is transgenic technology (*see* Chapter 17). This approach has the potential to shorten the time required to produce smolts as well as being able to produce them off-season. Fast-growing Atlantic and Pacific salmon have been produced using a growth hormone gene construct introduced by microinjection into eggs just before fertilization (Du et al. 1992; Devlin et al. 1994). The fast-growing trait is heritable, having been observed in F1 offspring. Further research in this field will include growth and metabolic studies, smolting biology, response to temperature and photoperiod, and to changes in salinity. A major consideration in the use of transgenic fish, where there is a possibility of escape, is that they must be incapable of interbreeding with normal fish. One method for achieving this is through the production of all-female triploids. Although triploids are used for commercial rainbow trout farming, the technology is still under development for Atlantic and Pacific salmon. One aspect of triploidy requiring further evaluation is its effect on smolting (Boeuf et al. 1994*b*).

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## Chapter 9

# REARING SALMONIDS TO MARKET SIZE IN MARINE WATERS

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## INTRODUCTION

The current global production of all marketed farmed salmonids is about 700,000 tons (t), of which 300,000 t are trout of various species farmed in fresh water. An insignificant tonnage of both Atlantic (*Salmo salar*) and Pacific (*Oncorhynchus* spp.) salmon are farmed in fresh water to market size. Thus, the remaining 400,000 t (57%) comes from more than 1,000 farms sited in marine and estuarine waters. Indeed, the expansion of farmed salmon production from a few thousand tons 15 years ago to the current level would have been most difficult, if not impossible, if grow-out production had not been able to move to the sea (Nash 1995) (*see* Ingram 1988 for a review of trout production methods and Gall 1992 for further review of rainbow trout biology and production).

Three basic factors made this expansion possible. First, was the availability of environmentally suitable, sheltered and partially exposed marine and estuarine waters in the middle to higher latitudes of both hemispheres. Second, was the permission to introduce or expand production of Atlantic and Pacific salmon for private confined farming in the many countries now involved in farmed salmon production. And third, was the technological development of floating net-pen systems to provide a confined environment for grow-out to market size. Other important factors will also be discussed in this section. As 80-90% of the salmon farmed in marine waters are Atlantic salmon, and approximately 10-20% are two species of Pacific salmon, namely the coho (*Oncorhynchus kisutch)* and chinook (*O. tshawytscha*), this section will focus attention on these three species.

## ENVIRONMENTAL AND PHYSICAL SITE REQUIREMENTS

### *General Conditions*

In order to develop an economically successful farm operation, the marine grow-out site chosen has to be environmentally suitable for the species desired, or, in some cases where a decision has been made to develop a site already in hand, a species may be selected that will perform well in that particular environment. Examples of the latter case are coho salmon farmed in the warmer waters of northern Honshu Island in Japan, and Arctic charr (*Salvelinus alpinus*) farmed in the colder waters of northern Norway and the very northern maritime provinces of Canada.

It is generally agreed that choice of site is one of the most fundamental aspects of aquaculture strategy and it often determines profit levels. The ideal site will vary from region to region and will depend on the species selected. There are also economic factors to be considered. Examples are distance from markets and various services, crew support, and general transportation costs. Published guidelines exist for most regions (for example, Caine et al. 1987; Pennell 1991*a*).

### *Seawater Temperatures*

The lower seawater temperature limits for Atlantic, coho and chinook salmon are about −0.7°C (the freezing point depression of salmon blood). Marine salmon farms in the Canadian province of New Brunswick, and the adjoining U.S. state of Maine are located in areas where the seawater temperatures rarely drop below 1-2°C during the coldest months. However, under conditions of prolonged, extremely cold Arctic winds, local seawater temperatures can plunge to −1 to −2°C, which is known as "super chill". When this occurs, the slightest stress (even feeding) can cause the blood to crystallize, stopping the heart (Saunders 1987; Boghen 1995). Apparently, mortalities are much higher in market-sized fish than in young post-smolts of the first winter in the sea. When "super chill" mortalities in marketable fish occur, they must be harvested immediately, which in itself can cause logistical problems. Since the fish are already at freezing temperatures in the water, super-cold wind chill factors during harvesting may be so severe that the harvested and exposed salmon may have to be protected from freezing during delivery to the processing plants.

Prolonged periods of low temperature (for example, below 4-5°C) will also have a negative effect on growth and hence farm production. Areas with winter sea temperatures of 8-10°C will show significant winter growth and much better annual productivity.

The upper seawater temperature limits for these three species are between 21-23°C, with chinook salmon less tolerant of these temperatures than coho or Atlantic salmon. Salinity is crucial to upper temperature tolerances, and as salinities rise above about 30‰, upper temperature tolerances drop dramatically with minor incremental rises in salinity. For example, coho salmon were raised to market size in Puget Sound waters (Washington state) with low mortalities due to environmental stress when summer salinities were 28-30‰ and water temperatures peaked at 17-18°C (Novotny 1975). But, when attempts were made to raise the same species in the Rade de Brest (Bretagne, France) in pure oceanic water of 33-34‰ salinity, mortalities due to environmental stress approached 80-90% as the water temperatures rose to 18°C (Novotny 1976).

The ideal seasonal sea temperature range for farming these three species of salmon is about 6-15°C. This range combines an optimal environment for maximizing growth while minimizing environmental stressors. These ideal conditions exist in many regions currently used for grow-out farms, such as central and northern Puget Sound, central and northern British Columbia, some regions of the U.K. and Ireland, the Faroes Islands, Norway, southern Chile and New Zealand. Environmental stress from daily fluctuations in water temperature is generally not a problem. However, in those areas where tidal currents could bring in waters that have been warmed in shallow bays or estuaries, other factors may affect production, such as increases in the numbers of pathogenic bacteria or parasites.

Farming salmon is possible under extreme conditions, although not advisable. The sheltered marine waters of northern Honshu Island (Japan) are far from ideal. Summer sea water temperatures reach 18°C by June and rise above incipient lethal limits to 23-25°C by August. How is it then that more than 400 farms were able to produce a peak production in excess of 25,000 t of coho salmon under these conditions? In essence, farmers worked around this problem by purchasing the earliest eyed eggs available, and accelerating growth in warm fresh water to produce a late zero-age smolt graded at 120 g by October for a late fall entry when the seawater drops below 18°C. As winter sea temperatures in this region (Miyagi Prefecture) rarely fall below 9°C, accelerated growth can be continued, and final harvests of 2-3.5-kg fish are completed when the seawater temperature reaches 20°C in late July. Economics, however, are eventually the determining factor, and the costs of raising coho salmon under these conditions began to exceed the costs of importing high quality farmed coho and Atlantic salmon from Chile and Norway. Consequently, coho salmon production in Japan has dropped precipitously since the 1991 peak.

### *Salinities*

The salinity of salmonid blood plasma is between 9-10‰, about the same as other animals, including humans. The ideal theoretical salinity then would be about 9-10‰, as the fish would be entirely isotonic with the environment, and would have to expend little energy to excrete excess salts, thus maximizing available energy for growth. This salinity environment exists in certain areas of the Baltic Sea, for example, and some farming of salmon and trout has been conducted there, but other environmental and economic factors have limited development in this region. In reality, if the three species of salmon discussed are properly smolted and conditioned when transferred to seawater, they will do best at sustained salinities of 32-35‰ when water temperatures are in the preferred range. This is especially true of Atlantic salmon (Laird and Needham 1988; *see* Chapters 7 and 8).

Smolting and various techniques for conditioning salmon for transfer to seawater are discussed in Chapter 8. In general, the three species discussed here can tolerate sustained salinities up to 21-22‰ without difficulty at almost any size within their preferred temperature range. Pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) can adapt to full salinity seawater as early fry, and grow rapidly when cultured in sea cages. Unfortunately, their market value is low, and it is rarely economical to grow them in the sea for the retail market. In the open sea, salmon are normally found in waters with a salinity range of 32-35‰, and these levels are found in most farming areas. The salinities of the majority of current operational salmon farms in both hemispheres are probably in a range of 30-34‰. Salinities of waters in inland seas, sounds and fjords where farms are heavily concentrated will vary seasonally and even daily, depending on flushing rates of nearby rivers. Salmon can tolerate substantial changes in salinity within a wide range, but rapid fluctuations may stress fish. This will not be desirable if it puts fish off of their feed temporarily, or negatively affects other environmental parameters. For example, infectious sea lice do not tolerate low salinities, but fouling mussels and some highly infectious parasites such as *Costia* sp. thrive in estuarine environments. In regions that have fjord or fjord-type environments, some growers have been able to pipe fresh water by gravity to floating raceways or net pens with plastic or rubberized liners. This has the advantage of increasing freshwater hatchery capacity. Young salmon can be introduced to a low salinity environment and grown through the smolt stages with little of the physical disturbance and stress that is associated with transporting smolts from typical freshwater hatcheries (*see* Chapter 12). Salinities are adjusted by manipulating the liners to allow lesser or greater intrusions of salt water. The disadvantages to such systems are that freshwater supplies usually originate in lakes or streams rather than wells, and the risks of infection from ubiquitous parasites and pathogenic bacteria or viruses must be given serious consideration.

### *Currents and Water Depths*

Marine cage or net-pen culture is dependent on currents caused by tidal fluctuations to provide a flow of oxygen-bearing water through the pens, and to sweep away or disperse as much of the faecal waste that drops through the pens as possible (Caine et al. 1987; Needham 1988; Pennell 1991*a*). At any given temperature, seawater has less dissolved oxygen carrying capacity than fresh water (*see* Chapter 6). Stress levels in seawater are such that most salmonids should be in waters that are at least 5 mg/l dissolved oxygen and preferably higher. The ideal net-pen sites would be moored in areas that experience back eddies during normal slack tidal current stages (time of tidal change), thus providing at least some flow at all times. All currents at a site should be studied carefully, as some current patterns can frequently bring "used" water back through the pens, which is certainly not a tolerable situation. Some of the most valuable investigations into proposed sites are studies of available data on tidal cycles and currents over a full year span. Although Atlantic and Pacific salmon can swim in the pens under almost any current conditions, the pens themselves will distort, reducing pen volume and possibly injuring fish. The period of greatest calculated or observed tidal current during a year should not exceed 0.6 m/sec. Some modern equipment may withstand higher current speed, possibly up to 1 m/sec.

Water depths should be based on the lowest tide of the year, and on the shallowest side of the net-pen complex. The minimum depth should be 10 m and frequently a minimum ratio of 3:1 (water depth to pen depth) is used (Needham 1988). If there is no movement of water during slack tides, the fish themselves will establish some current as they generally swim in schooling circles around the pens or cages except when feeding (Sutterlin et al. 1979; Chacon-Torrer et al. 1988). Periods of no water exchange from currents should not exceed one hour, and fish should not be fed during slack water. If slack water problems seem unavoidable at a site, then planning may require a reduction in fish density from the norm. Large farms should have hydrographic equipment to continuously monitor at least temperature and dissolved oxygen. It is important to monitor these conditions to establish records, not only to anticipate future conditions but also to have available environmental data in case of a fish kill. For example, in Puget Sound summer phytoplankton blooms and water movement can cause greater variations in dissolved oxygen than the differences caused by consumption of dissolved oxygen within the pens by the fish (Novotny 1980). Phytoplankton produce oxygen as a result of photosynthesis during the daylight hours, but they consume oxygen during the night. Unfortunately, there can be a serious lag time, and the period of peak oxygen consumption may not be during the night, but at 8 or 9 o'clock in the morning. The first feeding in the morning might have to be a light one at certain times of the year. Similarly, peak oxygen production might not occur at high noon, but possibly just before dark. Thus, the last and possibly the heaviest feeding might be just before dusk. This is a good example of the need to frequently monitor key environmental variables, and on large farms, investments in automated monitoring and recording systems are very worthwhile.

Sites with coarse rocky or muddy bottoms should be avoided. Not only is anchoring of the pen system difficult, but faecal waste can pile up between rocks, and on muddy bottoms it resists being swept away. Muddy bottoms have the additional problem of providing an ideal medium for hydrogen sulphide-producing bacteria ($H_2S$ is toxic to fish), and the addition of faecal waste compounds the problem. The ideal bottom is sand or gravel, both for anchoring and for waste considerations. Anchoring systems themselves can be quite complex, depending on the size of the system, predicted wave heights, and currents. Almost all systems with more than one cage joined together have multiple anchors at critical points. The greater the number of cages joined together as one array or "flotilla", the more complex the anchoring system becomes because the forces of wind and currents must be related to the sum of the parts. All calculations must be made for maximum adverse conditions, including fouling of the net pens. Fouled net pens can increase the drag coefficients by at least a factor of ten (Wheaton 1977; Needham 1988; and *see* Table 1). Farms with access to large areas of protected water can spread out large, single cages with one anchor per cage, allowing the cage to swing on the change in tide. This can provide good exchanges of water within the pens and dilute waste over a broad area. The anchoring mechanisms must be substantial and secure; with a single point anchor, there is no reserve if a cable parts or an anchor drags. In some regions, there has been development of exposed offshore sites subject to storm force winds, large ocean waves, and strong currents. Individual cages designed to withstand such adverse conditions (e.g., PolarCirkel™ and Ocean Spar Net System ™) are huge, exceeding 30 m in diameter, with volumes up to 12,000 $m^3$. They are designed to ride with the waves, much like strands of kelp, and because they are large and subject to both waves and current, they require sophisticated anchoring systems. Most offshore cages must be serviced by boat, as peripheral structures to allow storage of feed and support of staff would be prohibitively expensive to build.

### *Toxic and Destructive Phytoplankton Blooms*

Phytoplankton blooms kill many thousands of fish each year in sea cages throughout the world and although many species of damaging algae have been identified, there are many more yet to be discovered. Furthermore, the mechanism by which these organisms kill fish is frequently not well understood (Dale 1987; Okaichi et al. 1989; Black and Gowen 1988; Martin and Wildish 1990; Shumway 1990; Forbes 1994). Under some oceanographic conditions dying phytoplankton may kill or stress fish through dissolved oxygen reduction in the waters flowing through farms.

In general, sites that have poor flushing, poor currents, or excessive riverine influence may have problems with phytoplankton blooms. However, salmon farms at any coastal site may encounter harmful phytoplankton, and this may be difficult to predict. Two types of phytoplankton cause most of the trouble: (1) phytoplankton that produce toxic substances in the water; and (2) phytoplankton that cause physical injury. A typical example of the latter is *Chaetoceros concavicornis* (and congener *C. convolutus*), a large diatom with sharp silaceous, spiculated spines that can mechnically irritate gill filaments (Rensel 1994). When concentrations reach several thousand and more per litre, the gills become clogged with mucus and diatom spines, and abraded. As concentrations increase, gill hyperplasia, hemorrhaging, anemia, and mortality increase. Young salmon are most susceptible, as their gill filaments are more fragile than those of adults. Susceptibility also varies with species. Rainbow trout (*Oncorhynchus mykiss*), for example, are very sensitive to *C. concavicornis*, which is one reason why they are no longer grown on British

TABLE 1

Incremental weight gains due to marine fouling on various materials suspended in a coastal marine environment in Scotland. Gains are expressed as the percentage increase from the relative starting (clean) weight (adapted from Wheaton 1977).

| | Relative Weight/m$^2$ | | | | Percent gain | | |
|---|---|---|---|---|---|---|---|
| | **Clean** | **Jul.** | **Sept.** | **Nov.** | **Jul.** | **Sept.** | **Nov.** |
| Polythene (standard) | 1.77 | 5.3 | 200 | 356 | 199% | 11,199% | 20,013% |
| Courlene | 1.96 | 5.9 | 169 | 249 | 201% | 8,522% | 12,604% |
| Ulstron | 3.33 | 10 | 216 | 370 | 201% | 6,386 | 11,011% |
| Polythene (Copper proofed) | 1.77 | 3.5 | 80 | 168 | 98% | 4,420% | 9,391% |
| Netlon | 3.33 | 6.7 | 123 | 163 | 101% | 3,594% | 4,795% |
| Plastabond | 31.87 | 55.8 | 350 | 446 | 75% | 998% | 1,299% |
| Galvanized Weldmesh | 33.34 | 43.3 | 50 | 117 | 30% | 50% | 251% |

Columbia sea farms. The first reported incidence in the U.S. (Puget Sound, Washington state) more than 20 years ago caused a 95% mortality in pen-reared coho salmon post-smolts in 72 hours. There are many other *Chaetocerous* diatom species with and without barbed silaceous spines (that is, spines with spicules) which are at least candidates as agents of gill damage (Gaines and Taylor 1987).

*Heterosigma carterae* (formerly *H. akashiwo*) is probably the most common of the toxic phytoplankton, and has caused serious losses to pen-reared salmon in Washington state and British Columbia (Black 1991). These organisms can reach extremely high concentrations; Fraga (1988) reported a bloom of *H. carterae* of 18 million cells/l in 1987. This caused the first reported mortalities of cultured fish (sea bass) in Spain from plankton blooms. Toxicity of the dinoflagellate *Gyrodinium aureolum* to encaged cultured fish was reported in Norway as early as 1966, and subsequently in 1976, 1981, 1982, 1985 and 1987 with considerable mortalities (Dahl 1988). Okaichi (1988) reported losses in the summer of 1987 of cultured yellowtail (*Seriola* spp.) in Japan of 1.5 million from *Chattonella antiqua*. Marsh (1988) reported that British Columbia had severe losses of farmed salmon and trout in sea cages in 1987-88 from a number of phytoplankton, mainly by *C. concavicornis* and *H. carterae*, but *Pyrodinium* sp., *Gymnodinium* sp., and

*Distephanus speculum* were suggested as candidate problem species. There are many other examples of large blooms of hitherto minor, unknown algal species causing great harm or threat to sea-cage industries (for example, *see* Saunders 1988). These organisms and many other harmful species are not unique to the northern hemisphere; many are cosmopolitan. Toxic phytoplankton blooms have been reported in the salmon-growing region of Puerto Montt and Chiloe Island in Chile. Toxic blooms are not just recent events. The first author has had personal communications with biologists who recall seeing "clouds" of plankton pass through fish traps (pen-like devices that were once used in marine waters to capture migrating wild salmon) more than 50 years ago in British Columbia waters, and finding the entrapped salmon dead the next day.

There are no "cures" for plankton blooms. However, as they are primarily a surface phenomenon, and as they can move through an area in a matter of hours, some preventive measures can be taken (Shiroto 1989; Black et al. 1991). The phytoplankton densities usually decline with depth, and some farms have gone to 15-m (or more) deep cages to allow fish a depth refuge. In regions where salmon cage farms have been established and where blooms have been noted, farm operators have jointly contracted for small aircraft to search for surface "clouds" and monitor movement. If there is evidence of a risk, tug boats are brought in, the cage systems are detached from the moorings, and they are slowly towed to a temporary safe area. This is an expensive operation, and only conducted in extreme situations. Remote sensing via satellite imaging has been suggested for detection of blooms, but this approach has not yet been systematically applied to this problem. Other measures include sinking cages and airlift systems designed to move plankton free water from depth to the surface (Black et al. 1991). Generally, stopping feeding during algal events promotes survival.

In some regions farm-based "plankton watch" programs have been mounted in which farm workers take daily plankton samples and report species present to a central registry, which then communicates the results to all other farms in the region. In this way early warnings may be achieved. After farms have been operating in a region for several years, patterns may emerge, and farms may be prepared for plankton problems at certain seasons. There is always, however, the surprise event sometimes involving a species hitherto unknown in the area. All of these measures show some potential for success, but none eliminate the serious risks caused by harmful phytoplankton blooms. Salmon farms may be considered as floating bioassays along a coastline, and some regions have proven largely unsuitable for cage culture because of frequent phytoplankton outbreaks.

### *Predation*

Open water floating culture systems are subject to predation, including human. Underwater predators include dogfish (*Squalus* sp.) sharks (which can chew access holes through the nets), some diving birds such as cormorants, which will try to spear fish through the mesh openings, and seals and sea lions, which will try to bite fish through the webbing. A general approach to reducing predator attacks is to surround cages with various types of protective netting. Dogfish attacks can be prevented by sewing an extra bottom of heavier mesh to the net cage. Seals and otters may be excluded by suspending large meshed nylon "guard" nets around the cages or cage clusters, sometimes with "guard" nets also under the bottom of the cages. The form of these "predator nets" is varied and many types of mesh are used. The first author recalls even using war-surplus submarine netting at one site in Puget Sound (Washington state). The use of outside predator nets is expensive and requires maintenance as these nets eventually become fouled and must be cleaned or changed. They are effective only if they are properly arranged with adequate spaces between the predator net and the cage.

In some regions seals present the greatest risk to captive fish. Seals are intelligent, and once they learn how to break into sea cages, they are very difficult to control. They may work in groups and can cause the loss of many fish through destruction of cages, allowing escape or causing extreme stress. Public concern frequently prevents the use of lethal force on marauding seals so that prevention becomes the main solution to the problem. In any case, killing marine predators usually results in their replacements moving quickly into vacated territory. For many years auditory seal scarers have been employed to discourage seals, but almost always seals have acclimated to these devices (Mate and Harvey 1987). This includes the use of recorded killer whale sounds, and various startling or unpleasant noises. Recently the use of very intense underwater noise generators has shown more promise. These devices may actually cause extreme pain to marine mammal ears and seem to prevent acclimation. They may also have undesirable effects on other species of marine mammals, especially cetaceans, and this may result in a curtailment of their use. Recently a nearly life-size model of a killer whale has been used with reported success as a seal deterrent (Morgan 1995). Time will show whether acclimation to this novel measure takes place.

Another common mammalian predator is the river otter. These animals are also intelligent and often work in groups. They can be excluded through use of the above described nets including a net riser (an extension of the sea cage netting a metre or so above the water level) sewn to a "top net" (a light netting stretched over the top of the net). On a few farms otters have been excluded by electric, low voltage cattle fence lines surrounding the cages. Mink may cause minor problems to some near-shore farms, but they are easily excluded by the above systems.

The most frequent predation from above comes from birds such as gulls and herons. Herons cause the most serious problems. Fairly small meshed netting is stretched tight along the rail systems, and a light-weight, large meshed nylon "bird" or top net is stretched over the top (the netting used to protect vineyards from birds is extremely light, and is used frequently on fish farms). This system keeps out overhead diving birds, reduces the ability of perching birds (herons) to prey on the fish, deters land and marine mammals from getting into the cages, and prevents fish from jumping out. Large cages may have a very large float tethered in the centre to prevent the bird net from sagging and ensnaring fish.

All predator prevention devices incur significant operating and material costs to the farms' production, but are necessary to ensure successful fish growth. Attacks by predators probably cause as much damage through stress on the remaining fish than through direct fish consumption by the predators, and attacks by the larger predators frequently have allowed fish to escape, thus exacerbating concerns over the impact of escaped farmed fish on local wild fish stocks.

### *Farm Accessibility*

Almost all marine salmonid farms involve some type of floating system, which means that most are only accessible by water craft. Careful considerations must be given to site selection to provide:

(1) Reasonable proximity to a dock or protected beach area. Some type of staging area is needed to assemble perimeter floats, rafts, small barges, feed equipment, etc., plus a safe dock or float for transferring personnel to the cage sites and smolts to barges or "well" boats.

(2) Reasonable proximity to a processing plant to handle harvested fish. This can be either via a "live" vessel transporting live fish in water to a shore-based processing plant, or fish partially processed (killed, bled, and iced) on site, transferred to trucks on shore, and then sent to processing plants for finishing, packing and shipping.

(3) The possibility of remote site housing for crews and crew families. In British Columbia for example, some sites are so remote that excellent floating housing has been provided for entire families, including daily boat transport for school age members.

## SEA CAGE SYSTEMS

### *In-shore and Near-shore Farms*

The basic marine cage consists of some type of perimeter structure from which an enclosed cage to hold fish is suspended. There are two basic types of cage systems: (1) floating, the most common; and (2) submerged, which is fairly new technology, and is primarily intended for waters not well suited to floating systems (such as frequent storm or ice regions).

The basics of a floating marine cage or net pen are:

(1) Perimeter floatation to hold the system at or above the surface of the water;

(2) An enclosure with sides and bottom, and suspended from the perimeter system. This can be cuboidal, hemispherical, cylindrical, or conical in shape, and made of some type of mesh material large enough to allow the passage of water, but small enough to prevent the escape of the smallest fish transferred to the pen. Almost all marine cages are made from soft, knotless nylon webbing; and

(3) A method of preventing the cage from collapsing on the enclosed fish during tidal current flows.

Figure 1 is an aerial view of a small floating farm in one of the deep, sheltered inlets of coastal British Columbia; notice the easy shore access via a floating dock, and the mixture of cage types. The smaller farms use a variety of materials including wood, steel and PVC pipe to form cage supports and perimeter walkways set over styrofoam or barrel floatation. The earlier designs of small cages and flimsy wooden frameworks, often set up in sheltered bays, have been supplanted by sturdier systems capable of withstanding at least moderate wave and current forces.

Figure 2 is another aerial view of a sheltered farm using nested octagonal cages; notice that some of the pens have a perimeter walkway, while others can only be worked from a vessel. Perimeter floatation for circular and octagonal cages is typically several layers of large diameter, joined, flexible plastic pipe. The flexible pipe floatation allows the cages to flex when subjected to wave action. A square cage frame, 16 m on a side, made of a double row of styrofoam-filled plastic pipe will typically weigh over a ton without the net pen.

Figure 3 is a water view of a larger inshore floating salmon farm in the sheltered waters of Puget Sound (Washington state). This system uses a rigid aluminum perimeter walkway as support for each net pen. The perimeter floatation is polyethylene drums of approximately 200 l capacity. The aluminum frames are joined to the central walkways by a type of pintle and gudgeon that allows the rigid frames to articulate with wind driven waves. Superstructure pipe-rail framing allows the net pen to be suspended high above the water line. This forms an approximately 1-m "jump" barrier which prevents even the heartiest of salmon from escaping. Overhead bird barriers include seine twine stretched back and forth to discourage birds from diving on bigger fish (foreground), to tightly stretched webbing (background) for pens holding smolts. The pens are held open against the currents by hanging sacks of sand or 4-l plastic bottles of sand from lines attached to the pipe-rail framing. This is probably the most common method of holding pens open. The automatic feeder (*see* Figure 4) floats above the water, and is serviced by a pivoting arm.

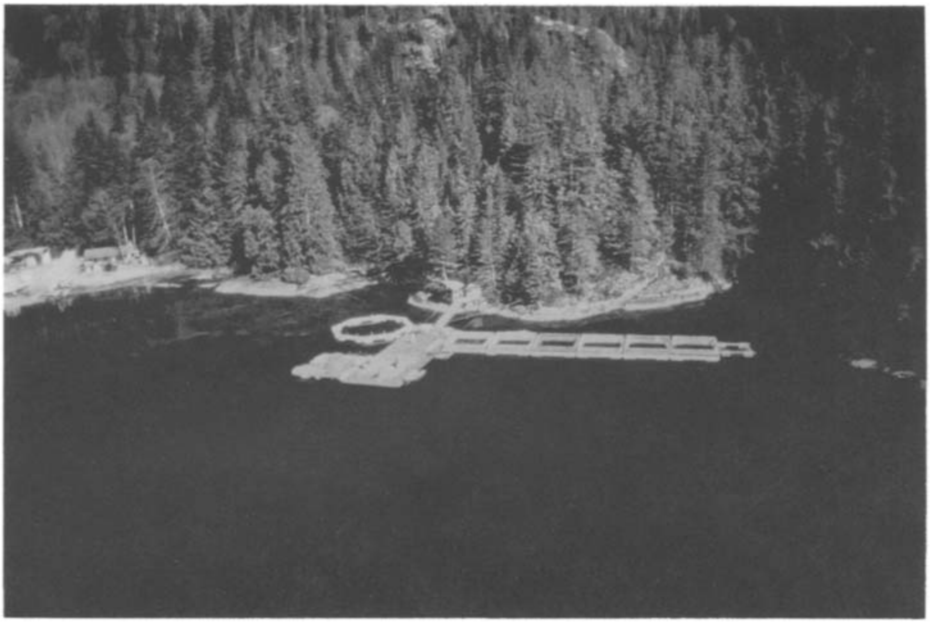

FIGURE 1. A small inshore floating salmon farm with direct beach access located in one of the many sheltered inlets of coastal British Columbia.

Figure 5 shows a large near-shore floating salmon farm that utilizes large diameter, styrofoam filled, fibreglass pipe for net-pen floatation. The cages are attached to central and outer articulated aluminum walkways planked with wood. Notice in Figure 5 the tight bird-netting covers and superstructure pipe rail framing made of lightweight polyethylene, and the feed containers on the decks. This farm uses hand feeding. Another large near-shore farm (Figure 6) utilizes all aluminum articulated structures, and high floatation. The automatic feeders on this farm are also placed directly over the pens, but in this case the feeders hang from the pivoting arm, rather than floating. A near-shore net pen complex of extremely large pens in reasonably sheltered waters on the west coast of Vancouver Island, British Columbia is shown in Figure 7. These pens are more than 20 metres on a side, and require mechanical equipment to move or adjust them and divers to clean out mortalities. It may become difficult to hold large-volume pens open against currents with inside weights, and it becomes necessary to use an alternate system, such as "down hauling". A common way to do this is to attach a pulley to a heavy weight at or below the depth of the pen. A long line is sewed into the outside bottom corner of the pen and passed through the pulley. This is repeated at strategic points along the bottom of the pen. When the heavy weights are lowered, the pen can then be pulled down by hauling the pulley lines. Although large net pens are difficult to

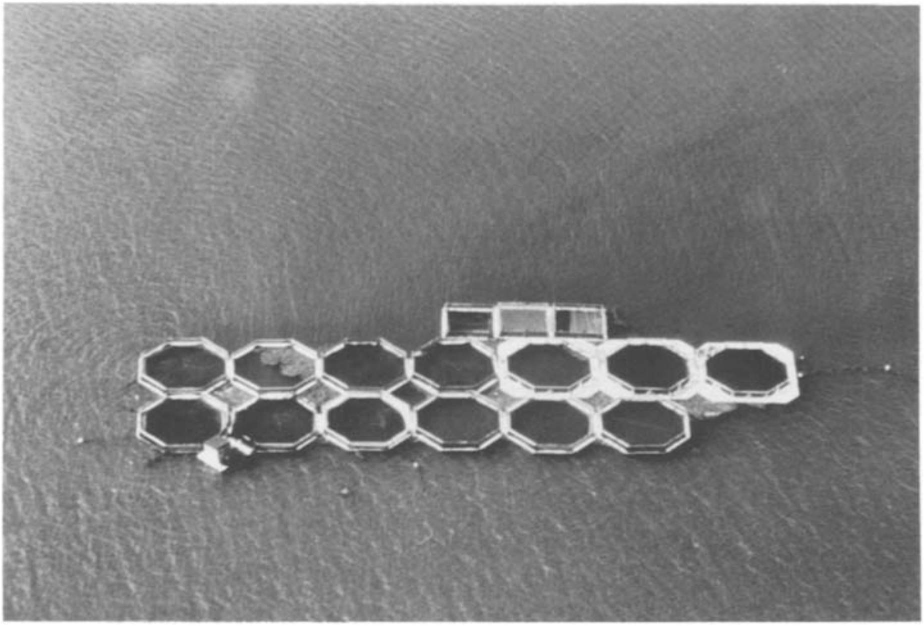

FIGURE 2. A small near-shore floating salmon farm that uses clustered hexagonal net-pens. This farm must be serviced by boat.

handle, the advantage is that the cost per unit volume of net pens drops as the size increases.

### *Off-shore Farms*

In-shore or near-shore sites are the most desirable for obvious reasons. Not many farm managers are enamoured with the thought of daily servicing cages floating in totally exposed off-shore environments, with high winds, monumental breaking waves, currents, and large vessel traffic. However, off-shore and exposed-site farming may well become more common in the future in some countries, as (1) the availability of in-shore sites becomes scarce, and (2) if moving off-shore become a means of escaping some serious diseases and predators. The technology exists and is being further developed. For example, Dunlop makes flexible rubber cages up to 1257 $m^2$ surface area, with two concentric floatation rings. Thus, a 15-m deep cage would have a volume of almost 19,000 $m^3$, and a 10-m deep cage would exceed 12,000 $m^3$ in volume. Helgeland Plast A/S of Norway makes a similar cage called the PolarCirkel™. These cages must be designed to ride with the waves, even up to force 7 winds, and yet not collapse on

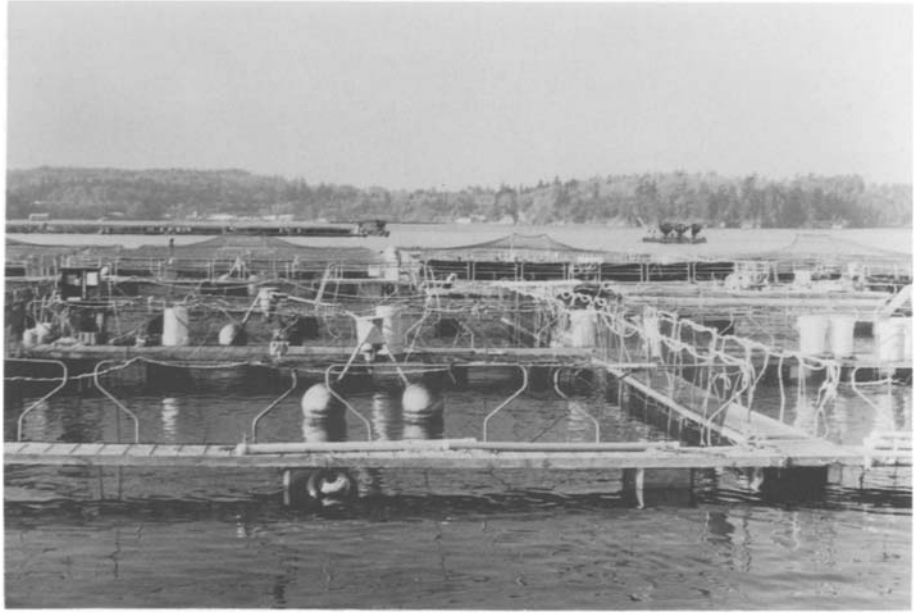

FIGURE 3. A large near-shore floating salmon farm in the sheltered waters of Puget Sound. The farm is serviced by boat and barge from the dock in the background.

themselves. Usually a single anchor is provided for each cage so that the systems may pivot with wind and tide. Anchoring is a critical matter and the anchors and rodes are very substantial. For example, I.C. Trawl makes cage nets for Ireland up to 20 m per side and 10 m deep, with approximate volumes of 4,000 $m^3$. The nets for these cages weigh 3.5 t prior to installation, and 5 t when treated with antifoulants. They present enormous drag when exposed to tidal currents.

The Ocean Spar Net System™ made by Net Systems, Inc. utilizes anchor-tensioned vertical floating spars at each corner to support large rectangular or square net pens. Thespars will "bob" vertically in the water with large waves, much like a navigational buoy, but they always keep the net pen above the water line. Servicing, as in most off-shore systems, is done by boat.

International interest is growing in the use of large, almost self-supporting steel cage systems for off-shore farms. For example, Campbeltown Developments, Ltd. has built steel Cruive systems for Landcatch Loch Fyne Farms' storm-exposed off-shore sites. They currently produce 1,000 t per year from two 8,000-$m^3$ Cruive off-shore steel cages, and they will expand to four units and 2,000 t per year. This is about 62.5 kg/$m^3$ of

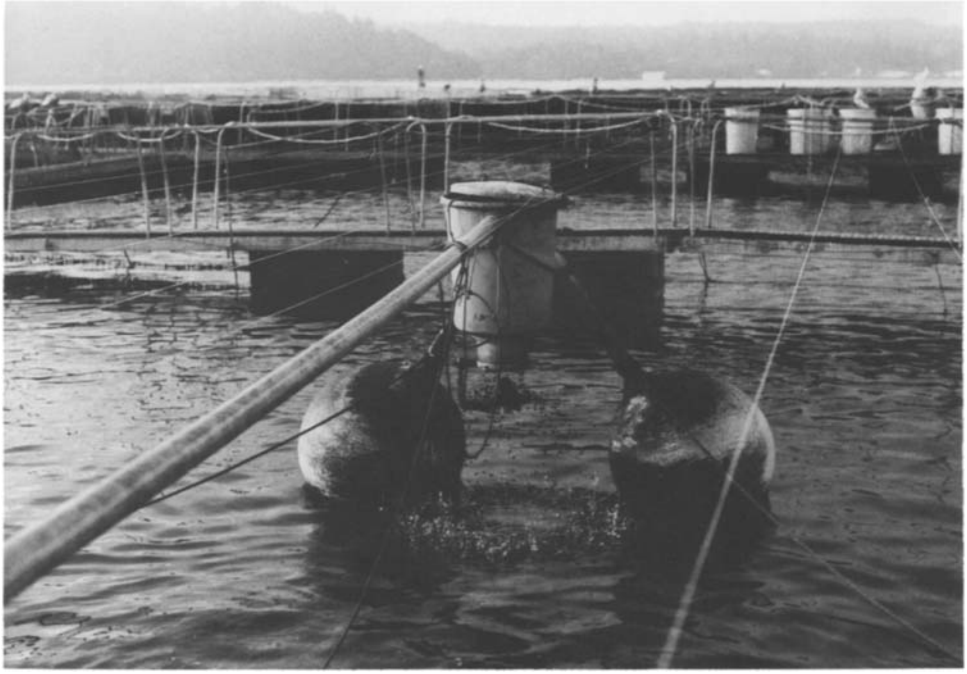

FIGURE 4. A floating automatic feeder inside a net-pen. The long pipe pivots to bring the feeder up against the perimeter walkway fo servicing. The lines strung across the cages are to deter birds.

rearing volume. Each two-unit farm can be operated by just two persons, and uses an automated, centralized feeding system which operates even during bad weather. Less than 10 years ago, seven to nine persons were required to produce 1,000 t of salmon in Norway, and final rearing densities were far less than those seen today.

A French and Norwegian consortium has converted an oil tanker into a pumped water off-shore farm that operates off the Brittany coast. It has a capacity of 17,200 $m^3$, and produced about 750 t the first year, or about 43.6 kg/$m^3$ (Novotny and Nash 1995). It has been estimated that shore-based farms (pumped water) might need to produce 60 kg per $m^3$ of pen volume to be profitable, and with this system more than two persons are required to produce 1,000 t of salmon. Thus, it appears systems such as the Cruive

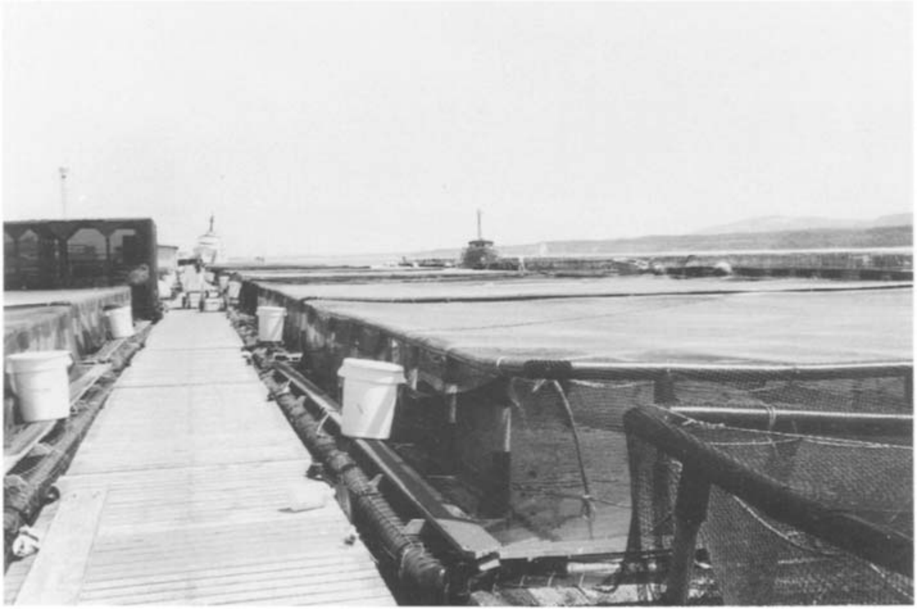

FIGURE 5. A large near-shore floating salmon farm that utilizes large diameter styrofoam-filled fiberglass pipe for net-pen floatation. The cages are attached to central articulated walkways. Note the tight bird-netting covers and superstructure pipe rail framing made of lightweight polyethylene.

may show great promise for large off-shore operations that require economy of scale in order to be profitable.

### *Pumped Water*

The desire for more control over the environment in marine farming is usually at odds with the situation of fish in open cage systems through which the environment essentially flows uncontrolled. As noted, damaging phytoplankton may be a major problem and may eliminate many otherwise good sites from sea farming use. Predators are a frequent difficulty, and it is difficult or impossible to manage treatments in open pens. A recent research area has been the use of tough fabric cones and low hydraulic head pumped seawater to create floating, solid-walled fish containers in place of traditional netting. The container then acts as a circular tank of large dimensions (*see* Chapter 6), and water may be pumped from a selected depth at relatively low cost due to the low head. Fish waste and dead fish may be removed via the bottom outlet and if

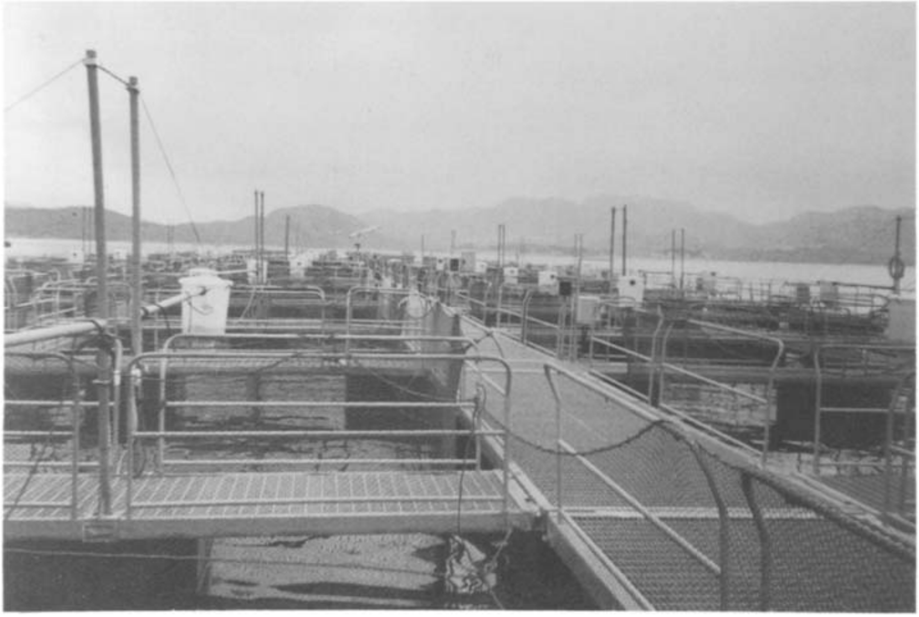

FIGURE 6. A large near-shore floating salmon farm that utilizes all aluminum articulated structures and high floatation. Note the variation in automatic feeder placement compared with that in Figure 4.

desired, waste can be concentrated for further treatment. Thus diving is eliminated, water flow and velocity may be controlled, waste is removed from the system, and deeper, hopefully algae-free water can be used during phytoplankton events. With such controls it is likely that higher rearing densities might be attained. All of these advantages must be balanced against capital and operating costs. Unfortunately little exists in formal publication about the performance of these systems, but there is a growing commercial literature which reports on the technology used and the results of current trials (C. Brentan, Aquahold Technologies Ltd., Nanaimo, BC, Canada, pers. comm.).

Similar rationales have been used in the past for on-shore rearing systems. These are large tanks, usually concrete, built on water-front land. Large pumping systems bring water onto the site from a near-shore intake and the farm is operated as any trout farm or freshwater hatchery. Performance has been good in such farms, but generally the expenses of operating and the capital outlay have proven too high to maintain profit over long periods. Most of the on-land facilities have ceased to exist although a few still operate with success.

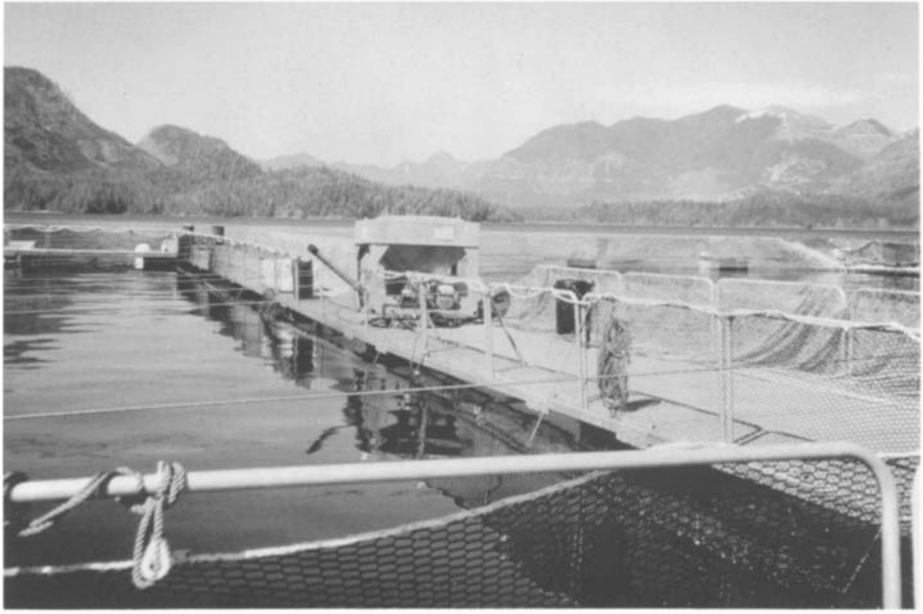

FIGURE 7. A near-shore net-pen complex of extremely large pens in reasonably sheltered waters on the west coast of Vancouver Island, British Columbia. These pens are over 20 $m^2$ and require mechanical equipment to move or adjust them.

### *Fouling*

The attachment of both micro- and macro-organisms to netting and floatation is a notable feature of all aquaculture, and sea cages with their very large exposed areas of netting including external predator nets, are greatly affected by fouling. Both plants and animals are common foulers. They affect sea cages by creating enormously increased drag, occluding water flow through nets, and by increasing the weight of nets suspended from floats (*see* Table 1). The degree and type of fouling varies with both region and season, and the frequency of net cleaning varies accordingly. If nets are not kept free of fouling, they may become so heavy that they cannot be lifted from the water (Figure 8). Even moderately fouled nets greatly increase labour involved in cleaning or changing operations. Fouling grows exponentially so that a slightly fouled net may become a highly fouled net in a short time. In addition to clogging nets, fouling has the potential of collecting fish feces and other detritus and becoming a reservoir for pathogens.

The main approach to the management of fouling is to change nets frequently. This incurs labour, but if it is done frequently the labour per net change is reduced and

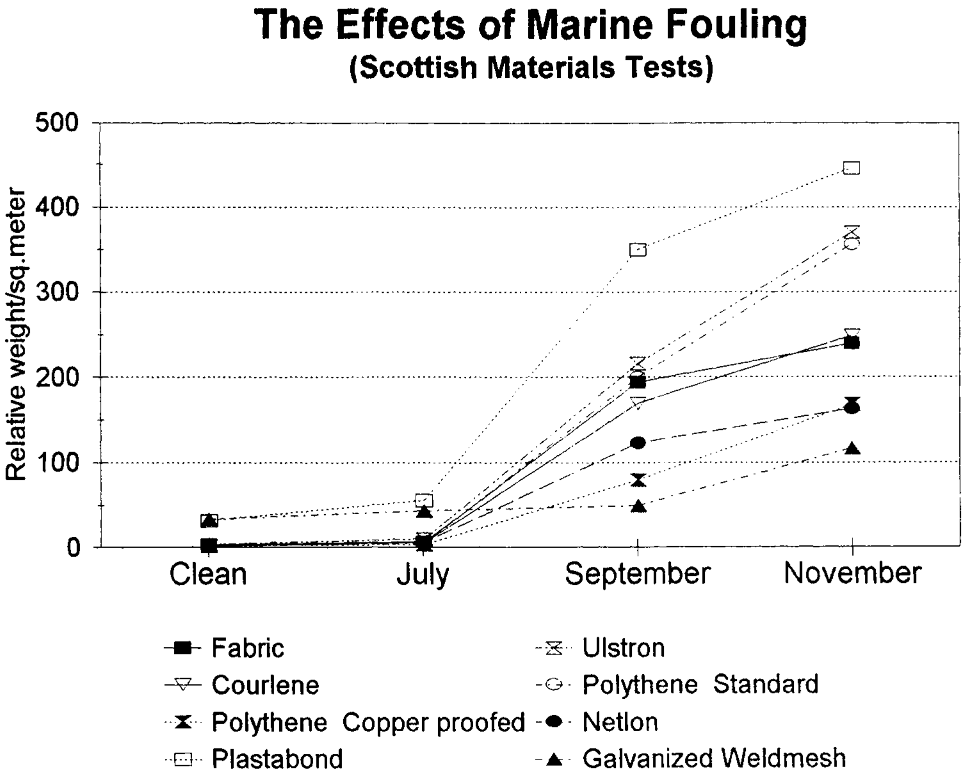

FIGURE 8. The results of a Scottish study on the effects of marine fouling on various materials suspended in a coastal marine environment, as measured by weight increases per m of net over time (adapted from Wheaton 1977).

the lightly fouled net can be quickly cleaned with a high pressure water spray or net washer. By contrast, over-fouled nets may be impossible to clean, and as noted, difficult to handle. The labour involved in net changes has led to experiments with a variety of antifoulants including copper and zinc compounds. Most commercial copper compounds work very well for a few weeks and then lose their effectiveness. There has been some concern that copper compounds could be toxic to fish; recently, however, some slow-release copper compounds are receiving renewed interest. Another anti-foulant, Tri-*n*-Butyl-tin (TBT) (and related compounds) has been available as a co-polymer which effectively releases this strong toxin steadily over prolonged periods by molecular erosion. This compound when applied to nets virtually eliminates the need to change or clean nets and was thus appealing at one time to salmon farmers. Various studies have shown, however, that TBT is a most dangerous toxin, is absorbed into fish muscle tissue, and has the potential to cause toxic effects in both fish and humans (for example, *see* Paul and Davies 1986; Short and Thrower 1987). It is now banned in most countries. Other antifoulants work on a low surface energy principle; various waxes, silicones, and similar smooth, slippery compounds can be applied to nets, making the attachment of the first colonizing fouling organisms difficult. It is claimed that such compounds also facilitate net cleaning, but they have not received much support from the industry and to date they do not appear to be very effective. The search for an effective, non-toxic antifoulant continues with new candidates each year; such a material would become a valuable addition to a salmon farmer's management tools.

The type of netting and the size of mesh affect the rate of fouling and the weight of fouled materials (Wheaton 1977; and *see* Table 1). Knotless nylon mesh is the industry standard, and unfortunately it presents more surface area for fouling than many other types of mesh, for example, knotted nylon. Larger mesh sizes present less attachment surface for foulers per unit area, but as noted above, meshes cannot be too large or fish will escape. There is also a tendency to use smaller mesh sizes to avoid entanglement of fish at the operculum. This ensures that fouling will always be a concern and expense on salmon farms. While toxic antifoulants are now either illegal or in disfavour, there are other approaches to reducing the labour involved in net changing and cleaning. Underwater cleaning devices are advertised in most trade publications. These can be used from the surface or by divers. Several firms, such as the Boris Net Company, make totally closed cuboidal net cages that fasten to special frames. Landcatch, Ltd. uses them off-shore in their "Smart" cage, which is 19 m to the side. The cube can be rotated in just a few minutes to expose one side to the air where it is allowed to dry. Spherical cages have also been designed that can be rotated to prevent fouling.

## HUSBANDRY PRACTICES

### *Feeds*

The salmon farming industry had a painful growing period over many years as feeds were developed for the marine environment. The first farms in Norway used fresh or frozen capelin (*Mallotus villosus*), ground almost daily, and fed by hand. Japanese trout and coho salmon farmers used a wide variety of fresh fish, usually caught in near-shore waters. Some farms in Japan still use these "wet" diets, and at least one Canadian firm still produces a fresh pelleted wet feed that is delivered several times each week to Canadian and U.S. salmon farms in the bays that border New Brunswick and Maine. Since live fish are 75-80% water, it is understandable that the food conversion rates just 12 years ago were higher than 11 to 1 in Norway (feed:wet fish flesh produced), and in 1983 it took 200,000 t of feed to produce slightly more than 17,000 t of salmon. The biggest disadvantages of wet feeds are that they have little or no storage life, they are too costly to ship any distance, and may be more polluting than pelleted moist, semi-moist or dry feeds.

Moist pelleted feeds contain about 40 to 50% dry matter, and have direct food conversions of about 2.5-3:1. These are made from fresh fish or fish processing waste mixed with various binders and vitamins (*see* Chapter 10) and must be used fresh or kept frozen. They are still used to a limited extent in Norway. Semi-moist feeds contain about 70% dry matter. The Oregon Moist Pellet, or OMP, is a well known semi-moist pellet developed for Pacific salmon hatcheries to replace wet and moist feeds, and was the first commercially produced semi-moist pellet. Dry feeds are about 90-95% dry matter and have direct food conversions approximating 1.2-1.6 to 1. The main advantages to dry diets are that they can be stored without refrigeration, they have a long shelf life, they can be shipped anywhere, and the pellets are less friable which makes them ideal for automated feeding systems. Pelleted dry feeds are dominant in Norway, and a typical generic formulation would be 45% crude protein (usually as fish meal), 22% crude fat, 15.5% carbohydrates, 2% fibre, 6.5% ash, and 9% moisture. Vitamin and mineral packs must be added to this also. As noted in Chapter 10, dry feeds may be simple compressed pellets ("steam pellets") in which case the upper levels of lipid will be about 16%, or they may be extruded pellets made by a different process allowing much higher levels of lipid (about 30-33% with present technologies). Fat composition may be altered as water temperatures change to improve performance (Novotny and Nash 1995). The use of high fat diets (digestible protein, DP, to digestible energy, DE, ratios of around 19) is a recent industry trend and is still being evaluated through research and industry trials. Such lipid-rich formulations appear to contribute to much improved growth rates and conversions (Alsted and Jokumsen 1990; Talbot 1995; Talbot 1996); it is interesting to compare these lipid levels to those of the wild diet (*see* Higgs et al. 1995*a*). Dry feeds now can be designed to meet almost any need.

Carotenoid pigments such as astaxanthin (now approved for U.S. consumption by the FDA) are used at various levels to produce the exact flesh coloration desired in the finished product. Skretting Inc. makes a feed to produce the desired amount of fat in salmon just for smoking. Feed conversions are improving each year, such that Hydro Seafood, the biggest salmon producer in Norway, plans to market 70,000 t of salmon in 1997, and reduce food conversions from 1:1.2 to 1:1. Thus, on a weight for weight basis, what once took 11 kg of feed to produce 1 kg of salmon may soon take less than 1/10th of that amount.

As the physical size of salmon farms expands and the number of off-shore farms grows, the demand will grow for feeds that can be stored on site in quantity and delivered automatically with minimum pellet breakage and production of "fines". For example, Golden Sea Produce in Scotland uses AKVAMARINA™ automated hose-feeding systems and barges that store 60 t of feed at a time. Thirteen sites produced 7,000 t of salmon in 1994 on about 10,000 t of food, a finished food conversion rate of 1.4 to 1.

The concept of using autolyzed diets (fish silage) pioneered by Professor Lauren Donaldson after World War II is still being tested on a commercial scale, but some day may be of great importance, especially in the North Pacific ocean where many hundreds of thousands of tons of scrapped demersal fish and processing by-product waste are thrown back into the sea each year. The basic concept is to grind the raw product, add water, acids and/or proteolytic enzymes, and allow the slurry to digest at a pH of 3.5 to inhibit bacterial contamination (the IPN virus can survive in fish silage at pH 4 for 4-6 months, and *Yersinia ruckeri*, the ERM bacterium, can survive for 12 days). Bones and scales settle out, reducing the ash content, and the slurry is neutralized for further processing and the addition of oils, vitamin and mineral packs. The use of autolyzed diets will be market driven, just as any other product.

Another area of research in formulated diets is the incorporation of canola/rape seed or soybean protein into pellets as replacement for marine and land-based animal protein (Higgs et al. 1994, 1995*a*, 1995*b*; Teskeredzic et al. 1995). When various anti-nutritional compounds and carbohydrates are removed from these seed and pulse meals, they appear to be excellent sources of dietary protein for salmonids. If the required protein concentration processes become cost effective, then land-based protein should become the dominant protein in formulated feeds. Salmon farming will effectively move several steps down the trophic ladder and become, in global terms, much more efficient and sustainable.

### *Feeding Strategies*

Feeding strategies are critical for a number of reasons. Marine farm sites are used either for grow-out to market, or for culturing brood stock, and a variety of strategies may be used for any one farm, depending on the desired goals. The optimum feeding strategy maximises growth and conversion efficiency while maintaining fish health and reducing stress. The quality of a farm's feeding strategy frequently makes the difference between profit and loss.

Approaches to feeding are discussed in Chapter 6, and while much of the theory discussed there applies to the grow-out phase, the cost implications are now much magnified since feed accounts for 30 to 50% of all operating costs. The cost and amounts of feed delivered to a large marine site are enormous and any improvements in utilization are bound to have positive economic and environmental effects. In the grow-out phase genetically selected stocks are predominant, and as the industry matures, increasingly domesticated fish will be reared. This may mean that in future, some of the intrinsic attributes of the wild fish discussed in Chapters 2 and 3 may have been or may become eliminated or attenuated. Thus research on intrinsic feeding rhythms of fish might apply less to farmed stocks than to wild stocks, for example, in a release hatchery. This possible uncoupling of the wild salmonids' behavioural and physiological heritage from the domesticated fish has not yet been well addressed by research. Feeding strategies focus on three main areas of concern: how much to feed per day or per week, periodicity of feeding, and mode of presentation of feed.

The primary considerations governing the size of the daily ration are water temperature and fish size, and feed manufacturers have now had sufficient experience with formulations and field trials to produce feeding tables for almost every species of salmonid cultured in seawater. Feeding tables, based primarily on feeding to satiation, are developed to give the grower expected growth rates for a particular formulation at different water temperatures and for different fish weights - smaller fish feed at a relatively higher rate per unit of body weight. As water temperatures drop, the feeding rate is reduced according to a simple regression formula. However, it is now possible to switch to formulations with higher fat content during the coldest periods to maximize growth. While higher rations could reduce feed:growth conversion ratios somewhat (but *see* discussion above and Chapter 6), most farms achieve maximum profit when growth is fastest, that is by reducing time to harvest. It must be noted that feed tables are at best a guide, and there are further considerations as discussed below.

Various factors including feeding and nutrition may affect the phenomenon of early maturation on salmon farms. When Pacific salmon such as coho and chinook mature a year or more earlier than the norm, the resulting smaller fish are called "jacks" and are usually male. Atlantic salmon maturing early are known as "grilse" and can be of either sex, but males usually predominate. Coho jacks are very small and have little commercial value. Chinook jacks and Atlantic salmon grilse are larger but only rarely are of suitable size for profitable sale, although in some cases the presence of anabolic hormones accelerates growth enough to reach harvest-size targets before actual maturation occurs. The size of these early maturing fish depends on strain and on the environmental conditions influencing growth, but rarely are such fish considered desirable.

The rate of early maturation is governed by several factors, chief among them being genetics (*see* Chapter 11), water temperature (for example, *see* Saunders et al. 1983) and feeding rates (Thorpe et al. 1990*a*). High feeding rate that results in accumulated lipid stores, especially mesenteric fat, appears to promote grilse formation. Reduction of lipid inputs a few months before maturation allows some control over the final proportion of fish maturing early (Thorpe et al. 1990*a*). The best farm feeding strategy will include considerations of overall growth rates in the sea-cage populations, proportions of grilse and size of grilse. Thus, overall, choice of site, genetic strain, and seasonal feeding strategy all influence the proportion of fish maturing at each age.

Feed manufacturers today produce complete guides for changing pellet sizes, and for determining feeding rates for each size of fish. When sampling of stocks in a pen indicates that the fish are approaching another pellet size level, it is best to start switching to the next feed size in increments mixed with the previous size. Even if the fish are graded for the new feed size, incremental change allows all fish to adjust with minimum wastage. Salmonids almost always prefer the soft texture of moist or semi-moist pellets, and have a more difficult time adjusting to a rapid switch to a dry diet, especially in salt water. Incremental changes will help make the adjustment with least feed loss. If it is not feasible to make incremental changes, a light spray of 1% fish oil by weight on the dry pellets just before feeding greatly improves palatability and acceptance.

The periodicity of feeding appears to be important. Programmable automatic feeders make possible an infinite number of feedings over the daylight hours, but the literature, much of it based on small freshwater salmonid stages, is somewhat unconsolidated in terms of the relationships between feeding frequency and both growth rate and conversion efficiency (*see* Chapter 6). With small fish very frequent meals may increase growth rates, and while this may also be true for larger fish, there is evidence that frequent meals may actually reduce both growth and conversion efficiency when compared to one or two meals per day (Crampton et al. 1991). Certainly it is plausible that frequent meals could induce forced stomach and intestinal evacuation causing lower assimilation rates, and if so the best way to feed would be by a smaller number of meals at intervals keyed to gastric evacuation rate which is a function of both temperature and fish size (Brett and Higgs 1970; Crampton et al. 1991).

Earlier, and also in Chapter 6, intrinsic feeding rhythms were discussed. Several recent studies indicate that farmed fish of various species do indeed display pronounced seasonal and daily changes in appetite and feeding (for example, *see* Thorpe et al. 1990*b*; Kadri et al. 1991; Thorpe 1994). This is also noted by many farmers, and on some farms feeding periods are keyed to apparent appetite. On other farms feed is delivered according to a schedule convenient to humans, or as noted above, according to fish gut evacuation rates. It is possible that feeding fish when they are showing an intrinsic increase in appetite may facilitate feeding to satiation of all members of the population. It is also possible that by feeding at these times better food utilization will result, but this has apparently not been well studied in fish. It may be possible to alter feeding rhythms by farm practice, and intrinsic rhythms can be modified by changes in the environment (Thorpe 1994). Certainly it is well-founded animal husbandry "wisdom" that best performance results from regular, predictable feeding activities on the farm.

A major challenge in feeding populations of farmed fish is to get the same amount of food into all individuals so that growth is as even as possible, reducing the need for grading. Even when salmon have been graded, some are more aggressive than others, and it is difficult to feed all fish in a population to satiation without wasting food. There are a variety of feeding methods used to reduce this problem and to maximize the amount of feed eaten. Smaller farms may feed their fish by hand, and by carefully observing feeding patterns, they can adjust the frequency and amounts of feeding to meet daily goals for each pen of fish. Some growers have found that they can feed a 7-day ration allotment in five or six days, and allow one or two days of fasting, with no difference in weekly growth or food conversion. This of course, saves labour. Demand feeders allow fish to feed themselves by nosing a probe or coloured paddle hanging at the surface in the centre of each pen. Demand systems may also be used in conjunction with automatic feeding devices so that fish trip an mechanised feeder which can be set to override fish activity for certain portions of a day. On larger farms hand feeding may be too labour intensive and automated feeders are often employed. These are usually operated by computer. Feed may be delivered to the cages via mechanical spreader, water jet, water pipe, air blowers or cannons, pipes, or overhead containers (Figures 4 and 6). It is most important to reduce the possibility of territory formation during feeding by making feed impossible to defend (*see* discussion in Chapter 6). This is best done by feeding across the entire surface of the pen simultaneously. This will have the added effect of reducing fish collisions during the initial feeding activity. The rate of feeding should be keyed to the rate of ingestion so that feed does not fall through the pen to the outside environment. The correct number of pellets passing the fish population per unit time further reduces competition for feed.

After the initial surface feeding frenzy is over, a large proportion of the feeding takes place below the surface, where observation is more difficult. If the farmer uses surface feeding activity as sole indicator of population satiation, then much of the population will likely be underfed, the less dominant fish getting the least feed. Some farms feed each cage twice with a short rest interval between feedings on the theory that there are essentially two populations in each cage, more dominant and less dominant fish. Feeding beyond visual determination of satiation (fish feeding at the surface) may incur over-feeding unless there is a means of keying feeding to fish behaviour deeper in the cage. While demand feeders are a possible solution, aggressive fish may trigger the demand feeder on instinct but with insufficient demand, and food is wasted. Furthermore, demand feeders may result in too many feeding episodes per day, or the setting up of territories within the cage.

Automatic feeders, while labour saving, may result in significant feed wastage unless the feeder can, in some manner, be tied to fish appetite. There is much recent work on this problem and several solutions have emerged. One approach developed by a Tasmanian company, AquaSmart P/L, is an adaptive feeder that has attracted great interest and may be especially successful in off-shore systems. The AquaSmart™ adaptive feeding system (AQI) monitors uneaten food detected by an underwater sensor and regulates feed input via a computer-controlled delivery system. The sensor mounts about 5 m below the delivery point and it detects and discriminates between faecal casts and pellets. Software regulated, the system dispenses a small amount of food in the morning. If no uneaten pellets are detected, the program increases the feed delivery rate. Extensive tests were conducted with the system, including 80-m off-shore PolarCirkel™ cages. The AQI is a sophisticated demand feeding system with much greater efficiency than typical demand feeders. Field trials show a 10-15% increase in growth, a 5% improvement in food conversion ratios, and a coefficient of weight variation at harvest of 15%, compared with a normal of 20%. Other similar systems are being tested using underwater video as the sensor of uneaten feed.

Another rather ingenious and simple system is the "Feedback System" of Moore-Clarke Ltd. (Nutreco). A cone is mounted into the bottom of the net cage and connected by airlift to the surface where the air-lifted water flows into a screen. As pellets are fed in excess they fall to the bottom of the cage, are swept into the air-lift system and appear at the surface on the screen. This signals the feeder that either satiation has been attained throughout the population or that feed is being delivered too rapidly. The latter case should not occur if there is knowledge of the population size and potential ingestion rate. Typically farms feed 20-30% more to their fish after installation of these systems, indicating previous underfeeding. The converse may be true as well and underwater feed detection systems may prevent significant feed waste. The determination of satiation point for larger fish in sea cages requires further study (D.A. Higgs, W. Vancouver Laboratory, Dept. Fisheries and Oceans, pers. comm.).

Constant and well thought out daily feeding regimes are important, but as mentioned above, dissolved oxygen may vary diurnally at some sites and during some seasons. Oxygen at each site should be monitored so that the heaviest feeding does not occur during low oxygen periods. Feeding should also be reduced during exceptionally heavy phytoplankton blooms and stopped altogether if harmful algae species are present or suspected.

As noted in Chapter 6, feeding behaviour and other aspects of the behaviour of salmonids in captivity has been neglected until relatively recently. It is now recognized that salmonids display complex behaviour in sea pens and that much of this is related to both feeding and stress (Sutterlin et al. 1979; Erikson and Alanara 1992; Smith et al. 1993; Huntington and Thorpe 1994). Several studies are now in progress using underwater video technology (Petrell et al. 1993; C. Groot, Pacific Biological Station, Dept. Fisheries and Oceans, pers. comm.), heart beat monitors (Floen et al. 1988; Juell and Westerberg 1993), and sonar systems in attempts to relate behavioral patterns to efficient feeding regimes and other farm activities (Bjorndal 1988; Ferno et al. 1988). These will likely be of significance to the efficient and healthy rearing of salmonid fish in grow-out facilities throughout the world.

### *Data Collection and Record Keeping*

When a salmonid sea farm begins operations, probably the most important advance preparation is laying out the procedures for data collection and record keeping. Fortunately, the microcomputers and software on the market today can make this economical and almost effortless. The basics that are needed are a word processor for report preparation, database software for keeping track of stocks and inventory control of feeds, costs, market prices, weather and water quality, and spreadsheet software for monitoring growth, food conversion, densities and mortality. Spreadsheets can also be used as databases, and frequently are. Spreadsheet models are used for predicting growth and density, and some of these are available through universities with aquaculture programs, private industry, some government agencies (Chase 1987, 1988), and the American Fisheries Society. Larger farms may develop or contract their own model programs, or modify existing ones to suit their needs. The major element in prediction modelling is the specific growth rate (SGR), a measure of the percentage body weight increase per day. Since the SGR varies with species, strain, water temperature, size, available dissolved oxygen, and diet, the model must use some standards to establish a database for comparisons (*see* Chapter 7 for a full discussion of the influences on growth). Standard data is available from a variety of sources (Bell 1986; Piper et al. 1982; Jackson 1988; Priede and Secombes 1988), much of it from freshwater systems.

The systematic collection and modelling of farm data is vital because it allows the farmer to detect changes in fish production and link these to potential influences which range from oceanographic variables to farm practices (e.g., densities, feeding strategies, feed brands). In many animal husbandry industries, producers send relevant data to a central office where is it analyzed, made anonymous, and then sent to all subscribers who can then gauge industry standards and the effects of various husbandry practices. This is often a quicker and less expensive means of improving production than formal research studies. Such systems are generally called Record of Performance Programs, and they have been instituted in some salmon farming industries. They are an extension of good farm-data management.

Salmon farms may specialize to meet certain markets or take advantage of local conditions and available fish stocks and species. To give some examples, Creative Salmon Ltd. in Barkley Sound on the west coast of Vancouver Island, British Columbia, grows chinook salmon to a large size, with specific fat content and colour goals set to match the Japanese market requirements. They base their growth projections on their temperature range of 5-15°C. George Weston, Ltd., the largest salmon grower in North America, is considering using all-female Atlantic salmon in their north Vancouver Island farms, where the water temperatures range from 7-12°C. They will have to select from the fastest growing strains. They will lose the growth potential of males which get larger, but will avoid early maturation problems (grilsing). The Landcatch Loch Fyne Farms in Scotland uses some S1.5 year "super smolts", which are put into sea cages in January and February at a size of 350-400 g. The object is to produce marketable 3-kg Atlantic salmon by the end of the year. Tri-Gen Fish Improvement, Ltd. and Aquaseed Co. produce genetically selected accelerated coho salmon for marine farms. Zero-age coho average 2 kg after 12 months in the pens, starting from 25-40 g accelerated smolts. As described in Chapter 8, smolts can now be produced over much of the year spreading harvest times throughout the year and allowing specific market targets to be met. The genetic development of specific strains of salmonids must be tailored to a variety of grower needs.

### *Managing Mortalities*

When salmon are transferred from the freshwater hatchery to the marine pens, they face their first challenge for survival. If all transfer factors are favourable, that is, the fish are properly smolted or of suitable size for the season, have been given a clean bill of health as disease carriers, and transportation procedures are normal, there should be few mortalities. It is common now to achieve more than 80% survival from smolt introduction to harvest and at these survival levels there will usually be a profit (Needham 1991). Mortalities should be monitored carefully after the fish have settled into the new environment, which may take a few days. Smolts may work along the periphery of the pens for days, looking for a way out. Some will explore the netting, and if the fish have not been properly graded for the mesh size, some may wriggle through, as the smolts shrink slightly the first few days in seawater. If the skin is abraded, they become susceptible to myxobacterial and vibrio infections. The first author has observed that if this were a serious problem, heavy plastic sheeting could be arranged as a skirt around the inside of a pen (for 10-12 m square units) about 3 m down. This modification did not seriously interfere with water exchange, and the smooth plastic reduced the skin damage remarkably. The sheeting could be removed after the fish became accustomed to the pen.

In general, extreme care is required for the first few weeks after smolt introduction to saltwater cage systems (Pennell 1991*b*). At this stage, Atlantic salmon may be the most delicate of the major species reared in salt water, often requiring several days or more to resume proper feeding and to become used to the open and well-lighted environment of the sea pens. This is also a time when mortalities may easily go undetected, which among other problems, may cause the farmer to lose track of inventory. The fine-meshed smolt nets used at this time will foul very quickly (especially as many smolt introductions occur in the springtime).

Mortalities can be monitored in a number of ways. In small pens (10-15 m) less than 10 m deep, it is usually possible to vacuum smaller fish from the bottom. In larger or deeper pens, it may be advisable to have a "sock" sewn in the bottom of the pen (*see* Chapter 13). The sock can be connected to a flexible pipe to the surface. If the area around the sock throat is weighted slightly, any mortalities will collect in it, and they can be brought to the outside surface for collection by an airlift pump. Many farms use divers, and depending on the size of the farm, they may be working almost every day. The tendency in very large off-shore farms employing deep cages, such as the Cruive 20 metre system, is to use remote operated vehicles (ROV). At Landcatch Loch Fyne farms, Scotland, one operator from the surface can inspect and clear the mortalities from seven such cages in 3 h with a Manta™ 505 ROV.

Clearing mortalities is important, and the frequency of clearance will depend on what is happening in the pens. If mortalities are few and not increasing, they should be cleared at least once per week. Fresh mortalities, of course, should be examined closely.

If it is apparent that injury was not the cause, then they should be examined for disease. In the case that mortalities are increasing, removing dead fish must be done very frequently and if the mortalities are due to a serious infectious disease such as furunculosis or vibriosis, it is essential to clear daily. This is especially true of furunculosis, caused by *Aeromonas salmonicida*. This gram-negative pathogen can be carried by fish infected in fresh water, can survive in salinities at least to 32‰, and is transmittable at least 5 km in the sea. A serious aspect of this organism is that it proliferates in the carcasses of fresh, dead infected fish, and sheds enormous quantities of the live, infectious pathogen into the water. This is why clearing the mortalities is so important in the presence of this pathogen (*see* Chapter 13).

Under no circumstances should mortalities be thrown overboard. They should be collected and disposed of shoreside, either by burial or collection by reduction companies, or liquified at low pH using ensiling techniques. The latter technique has become the industry standard in some countries since it removes odour, pathogen load, is easy to handle and avoids various conflicts on shore (Carswell et al. 1992).

### *Stocking Densities and Grading*

Although grading is done fairly frequently in fresh water, it should be minimized in seawater, as the stress from handling in seawater can cause serious problems. Some farms avoid grading altogether, stocking the net pen with the required amount of fish to attain target densities at harvest. Others grade once or twice during production using the opportunity to reset inventory estimates and divide fish into separate pens to adjust density. In some cases fast-growing fish (often grilse) are harvested during grading. Grading in the huge off-shore pens is generally restricted to first harvest. In general fish should not be graded or handled until the water temperature drops below 10°C, but also not below 4-5°C (Needham 1988; Pennell 1991*b*). Some growers then grade at each next mesh size increase. If growth within a population is fairly uniform, it may not be necessary to grade until the first level of harvest.

Rearing density is discussed in both Chapters 6 and 7, and the general principles are the same in both fresh and salt water. High rearing densities may depress growth, increase stress levels and encourage disease, although mechanisms are rarely clear and actual rearing densities observed on farms vary greatly. Relatively little formal research on density has been done in sea cages due to the great expense of such experiments and the difficulty of designing experiments without confounding variables. Density will usually rise from a very low level at smolt stocking to a higher target level at harvest. This varies from as low as 10 kg/m$^3$ to as high as 60 kg/m$^3$. The normal range of harvest density is 20-30 kg/m$^3$. Weston's Vancouver Island farms use a maximum stocking density of 10 kg/m$^3$, even in their 30-m cages, but the success of vaccines for most bacterial pathogens has allowed some farms to operate at very high densities with apparent success, although there are dangers to this.

A common method of grading is to crowd the fish to one corner of the pen, and then pump them out with a fish pump or fish escalator through a grader with flowing water that sorts the fish into two or three pens. To give an example, some impellorless pumps can move and transfer 3,000 3.5-kg fish/h. A pen with 40 t of salmon could be transferred in about 4 h. Some pumps can move and grade up to 3,000 fish/h. A rotating Achimedes screw type pump is now widely used to move fish in pockets of water from pens to grading machine. These machines appear to do little damage to fish. Fish should be starved for 24 h before grading.

Inventory control, as noted above, is critical but has always been a problem in sea-cage culture, and grading is an ideal time to keep track of pen inventories. Fish graders may be coupled with various types of counting devices to determine how much of a discrepancy has occurred since the last enumeration due to unaccountable losses. Approaches to fish counting vary from simple hand counting by crew members, or video tapes of fish passing a white panel to be later counted by humans, to sophisticated computer scanning systems which measure and count fish as they go through a special passageway. All methods have potential for some error and this is often caused by a tendency for fish to move forward in hard-to-count groups.

In-water systems for inventory estimate have been proposed and designed for some years but only recently have moved into commercial use. Most systems use sonar to capture whole images of the entire fish population and software programs to sort this out into average fish size, fish number and resulting biomass. There have also been attempts to do this with video systems linked to computers (for example, *see* Petrell et al. 1993) or combined with tag recapture methodologies. One system measures mean size using a grid of light beams through which fish swim as they circle in the pen. This is a rapidly changing technology little represented in formal publications, but easily accessible through trade journals and papers. The advantages of *in situ* fish enumeration and measurement are obvious.

After 12 to 18 months in sea pens, fish are ready to harvest at weights ranging from 2 to 5 kg. Since much of the advantage to farmed salmon on the market lies in their higher quality, quality control on the farm is emphasized. This starts with a period of starvation prior to harvesting which reduces fat content in muscle tissue and the flora in the gut, and increases shelf life. Density in live transfer boats should be less than 160 kg/m$^3$, and stress levels should be kept as low as possible since stressing changes muscle glycogen levels, which reduces shelf life. Fish should (ideally) have 24 h of rest before killing to restore glycogen levels, or they should be transported at lower density levels.

### *Fish Health Management*

The most challenging problem in production farming in sea cages is preventing or treating infectious microbial diseases and parasites, and compared to cold freshwater environments, there is a plethora of disease and parasite organisms resident in coastal waters. New disease organisms are regularly appearing on farms throughout the world, although most of the serious disease and parasite problems have been known for decades. Treatments have become more difficult due to restrictions in drug and chemical use, but more research and development effort is being put into biologics, such as vaccines and antisera, than ever before. These efforts have proved successful in the case of bacterial vaccines, and have paved the way for larger and more profitable farmed salmon production. The three biggest problems are pathogenic bacteria, viruses, and parasites. The major fish diseases and parasite species of salmonids in captivity are discussed in detail in Chapter 13. Here, a few of the most common problem afflictions of marine salmonids on farms are briefly discussed in the context of husbandry and management.

*Pathogenic Bacteria.* The majority of disease problems in the northern hemisphere are caused by five gram-negative bacteria, and one gram-positive organism. The gram-negatives are three vibrios, which cause vibriosis, one that causes furunculosis, and one that causes enteric redmouth disease (ERM). The latter disease has not yet been a problem on marine farms although the bacterium can survive in salt water. All can be treated with some degree of success with antibiotics, but all can also be prevented or reduced by vaccination.

*Vibrio anguillarum* is the warmer water vibrio, and usually causes vibriosis problems in the late spring and summer months. It is common throughout the northern hemisphere. It is primarily enteric, as are a host of other vibrios, and might even deserve a title of "fish cholera", because if left untreated, it can decimate populations in a matter of days. Oral delivery of tetracyclines in the feed was, in the past, always the standard treatment. Now however, both immersion and injected vaccines have been so successful and so commonly used that antibiotic treatment due to epizootics is rare.

Vibriosis was seen as a summer disease in the early days of salmon farming, but, later it became clear that *Vibrio ordalii* and *V. salmonicida* (Hitra) were significant pathogens that frequently occur after water temperatures begin to decline in late summer. They can also be found at the same time as *V. anguillarum.* Thus far, Hitra disease has not been reported in Pacific salmon farms, and is common chiefly in Norway. These coldwater vibrios can be more difficult to treat as they frequently affect the head region and brain rather than the gut. However, vaccines are effective, especially the injectable vaccines, and the multi-valent injectables for fish larger than 25 g should be effective throughout the rearing cycle in all salmonid species.

Furunculosis is a devastating disease caused by *Aeromonas salmonicida*, and originally was a major problem in freshwater hatcheries in the northern hemisphere in all salmonid species. Atlantic salmon are especially susceptible to furunculosis. It is not transmitted via the egg or sperm, and with proper care of brood stock, it is possible to avoid contaminating a freshwater hatchery that uses ground or spring water.

As noted above, this organism can survive well in seawater, can be carried from fresh water to the marine site by infected fish, is transmittable in seawater over long distances, and sheds itself in great quantities from diseased mortalities. In addition, it can infect resident marine fish and treatment is difficult. This organism is resistant to many antibiotics. Once furunculosis has invaded a sea farm, the best way to eliminate it is to take all of the pens, perimeter floats, and other equipment out of the water, disinfect all materials, and fallow the site for at least one cycle. Fortunately, after many years of research and development, reliable vaccines have recently been developed that are now available to prevent or better manage furunculosis. The most successful have been the polyvalent oil adjuvanted injectable vaccines used to prevent furunculosis and vibriosis of all types in Atlantic salmon with one injection. The vaccines are injected when the fish are 25 g or larger in the freshwater hatcheries, and preferably before they have smolted. They are injected in the body cavity by vaccination teams or recently by machines developed specifically for this purpose. The costs average about $0.12-$0.15 per dose, including labour, and they are enormously effective. These vaccines were first introduced into Norway in 1992-93. As evidence of their effectiveness, Norway's use of antibiotics dropped from 6,114 kg in 1993 to 1,117 kg in 1994, while salmon production rose from 180,000 t in 1993 to 207,000 t in 1994. Vaccine doses rose from 12 million in 1993 to 23.3 million in 1994. More than 50 million Atlantic salmon are now injected each year in Europe, Canada and the U.S., and the procedure is accepted industry wide.

Enteric redmouth disease (ERM) is caused by *Yersinia ruckeri*, another gram-negative bacterium. It has been sporadic in its appearance, and apparently has not been a serious problem in sea-cage farms. This pathogen has been more of a problem in freshwater trout farms, but infected fish can become carriers to seawater. Immersion vaccines have been developed for this pathogen that are especially effective in fresh water. The pathogen normally responds well to antibiotics. It has been isolated from moribund juvenile Pacific salmon in sea pens several times and tests have indicated that it can grow on media with 3% salt (equivalent to 30‰ salinity).

One of the most difficult salmonid diseases, bacterial kidney disease (BKD), is caused by a gram-positive pathogen, *Renibacterium salmoninarum*. This pathogen has been reported in all species of Pacific salmon and Atlantic salmon in the northern hemisphere. Forty years ago, it was not known that the BKD organism is vertically transmittable in the egg, and BKD was thus introduced into Chile with Pacific salmon eggs. BKD can be (and is frequently) carried by infected smolts into seawater, and may not become pathologic for months or until fish are stressed. Although BKD pathology is commonly seen as gross lesions of the kidney, other organs can be damaged. It can be found in faecal casts, and may possibly be transmitted horizontally when fish in the net pens mouth sinking faecal casts. BKD is more frequently chronic than epizootic, but if mortalities reach 1%/day, then one-third of the crop will die in a month. There have been some reports of effective oral treatment with tetracyclines, but the most effective drug has been erythromycin. As is the case with most gram-positive organisms, vaccine development has been difficult, and there are no commercially available BKD vaccines on the market at this writing.

In the southern hemisphere, farmers in Chile have problems with another bacterial pathogen, *Piscirickettsia salmonis.* Beginning in 1989, it was the causative organism of mortalities in large farmed coho salmon in Chile, and within a few years it was a serious problem because it primarily effects fish just before harvest. This a very significant problem for Chile because it threatens the expected increases in salmon and trout exports (from 76,327 t in 1994 to 90,000 t in 1995, a 17.9% increase). The current production consists of: 28,000 t of coho, plus 35,000 t of Atlantic salmon, 26,000 t of trout, and 1,000 t of king (chinook) salmon. Chile is now one of the largest exporters of farmed salmon in the world, 60% of which goes to Japan, and 30% to U.S. Research into this pathogen continues; however, there are no vaccines available at this time (Anonymous 1995).

*Pathogenic Viruses.* There are a number of viruses that are pathogenic to farmed salmon in seawater pens (*see* Chapter 13), but the most serious are those causing infectious hematopoietic necrosis (IHN) and infectious pancreatic necrosis (IPN). The IPN virus is more prevalent in Europe. In the past, it has been primarily a problem in the freshwater hatcheries (for example, IPN is present in approximately 40% of the UK trout farms), but recently is has become a problem in Atlantic salmon in sea pens when it was introduced into this environment by infected smolts.

The IHN virus has only recently become a problem, and then mainly in Atlantic salmon on sea farms in British Columbia, even though the fish are not carriers when they go into the sea pens. Many of the farms in the north part of Vancouver Island are located right in the migratory path of sockeye salmon (*Oncorhynchus nerka*) returning from the open North Pacific Ocean during the summer months. Millions of sockeye pass through these waters each summer on their homeward journey, and some of them may be carriers of the IHN virus. It has been postulated that this may be one of the sources of transmission, as 4,000 to 6,000 t of Atlantic salmon are farmed each year in Puget Sound (Washington state) waters to the south, where IHN is not a problem, and there are few sockeye salmon. There is no treatment program for either IPN or IHN, but several companies are in the vaccine research and development stages for both viruses.

*Internal and External Parasites.* Compared to free roaming salmon, which depend on diets of crustaceans and fish, and consequently pick up a wide variety of internal parasites, farmed salmon, which subsist on pasteurized food, are relatively free of internal parasites, but there are several problem species both internal and external.

*1) Sea lice.* Parasitic copepods are the external parasites of greatest importance on salmon farms. These are the salmon and sea lice which have caused millions of dollars in damage in Ireland and Norway, and more recently have become a major problem to the salmon farming industry of New Brunswick. Sea lice in the Bay of Fundy have caused losses estimated at $15-$20 million (Anonymous 1995). The most important of these organisms are *Lepeophtheirus salmonis* (the salmon louse) and *Caligus elongatus* (the sea louse). These small crustaceans move about on the surface of the skin feeding on mucous secretions. In small concentrations they do little harm, but when many occur on one fish they begin to eat through the skin and may actually expose deeper tissues including the brain. Secondary infections or stress-mediated diseases may follow. Larval stages of lice are planktonic and they may drift into sea sites and attach to captive salmon (Bruno and Stone 1990). Some species of salmonids appear to have more resistance than others; chinook and coho salmon, for example, are more resistant than Atlantic salmon and rainbow trout. The warmer waters off the west coast Ireland are conducive to rapid salmon growth, but unfortunately they are also conducive to proliferation of sea lice. Even in the colder waters of Norway, they can cause reduced growth and losses up to 11% (Nygaard 1995). Elsewhere, losses may be greater, and many other regions have similar problems with these parasites (for example, *see* Shaw and Opitz 1993).

Eradicating lice may never be possible, but there are methods of controlling them and the search for more control measures is intense. This may be the most critical challenge to face the salmon farming industry thus far. Multi-faceted approaches will have to be used including site fallowing, baths, oral dosing, light traps, vaccination and cleaner fish (Anonymous 1995).

There are several available compounds for bath treatments; the most commonly used are organophosphate insecticides such as Dichlorvos (DDVP). These are generally efficacious but DDVP recently has shown some indications of a loss of effectiveness. Hydrogen peroxide, a more recent innovation, is very effective. It is available in 35 and 50% concentrations; the latter is slightly more hazardous to handle and is more expensive than DDVP, but leaves no residue and has beneficial effects on environmental bacterial loads. Paramove™ is a hydrogen peroxide disinfectant offered by Solvay Interbox, Scotland, that has had three years of success in that country. Hydrogen peroxide treatments offer only temporary relief. Azimethopos is an organophosphate with a potency of 10 times that of DDVP. It has an environmental half-life of 10-11 days, low toxicity to humans, and no tissue residues. It is much less expensive than hydrogen peroxide, and just marginally more expensive than DDVP. It acts very rapidly, and is most effective at higher temperatures and lower doses than DDVP. Cypermethrin is safe for humans to handle, controls all sea louse stages, and is effective at low doses (<100 ppb). It absorbs to solids of all kinds, including sediments, and is then rendered toxicologically inactive (Sommerville 1995). Other compounds continue to be tested; iodine has been used both as a bath and as an oral medication (Mustafa and MacKinnon 1993) but this is at a very preliminary level of investigation.

In Norway, tests were conducted with a mixture of garlic and onion pulp floated on the water surface. When the fish jumped, they would, in theory, expose the sea lice to the pulp and they would then drop off. At first it appeared to be successful, but after a number of trials it was shown that it really does not work (Nygaard 1995).

There are also oral treatments. Ivermectin™ has been used with good success, but has limited approval and various pryrethrum derivatives have also been investigated. Other new compounds are now being tested, some of which are highly effective against all stages of the sea lice (Sommerville 1995). Any ingested compound must be given approval by relevant government agencies both in the country where the salmon are farmed and in the country where the product is to be sold.

It is known that sea lice are attracted to light. Terecos, Ltd., of Glasgow, Scotland, makes Sea Lice Lure™. This system produces a unique light pattern underwater that mimics salmon scales. It then traps the lice and holds them for removal. A biological control, the sea wrasse (*Ctenolabrus* sp.) have been studied as cleaner fish (Bjordal 1992) and they are being used on some Norwegian farms. Although they are not 100% effective, they can keep louse populations manageable. The sea wrasse are susceptible to furunculosis, however, and it may not be advisable to use them if this pathogen is present in the salmonids. It is now permitted to use cunners (*Tautogolabrus adspersus*) as cleaner fish in North America (Roth 1995).

Fallowing is very effective if done within a wide area and for at least 6 weeks, preferably 10 weeks. Some farms in British Columbia prefer combinations of 9-17 weeks of fallowing followed by treatment at the first signs of sea lice.

A vaccine has been developed in Australia for cattle ticks (another type of louse) after 12 years of effort. This has encouraged developers of fish vaccines, and as a result of the Australian success, sea louse vaccine research is ongoing with renewed vigour.

2) *Other parasites of some note.* Kent and Margolis (1995) published excellent information, summarized here, on lesser known parasitic protozoans of salmonids cultured in seawater that could eventually cause problems for the farmers. These are also discussed in Chapter 13 (*see also* Heckmann 1993).

a) *Amoebae.* The only serious amoeba infestations in marine reared salmonids are caused by *Paramoeba pemaquidensis,* which infest the gills, and have caused upward of 25% mortalities . Infestations have been reported in Washington state, California and Tasmania. The best (safest) and most effective treatment is a freshwater bath.

b) *Ciliates.* The only reported ciliate to cause disease in salmon cultured in seawater is *Trichodina* sp., which is effectively eradicated with 1:2,000 or 1:4,000 formalin baths for 30 min before smolts are taken to sea pens.

*c) Hexamitids. Hexamita* sp. have infected pen-reared salmon in British Columbia and in Norway. *H. salmonis* in British Columbia is highly infectious and is easily transmitted in fresh water or seawater. Several drugs have been recommended, but none are being used commercially.

*d) Ichthyobodo (Costia). Ichthyobodo necator* is a common flagellated gill and skin pathogen in fresh water which can survive and proliferate in seawater. (Prior to marine culture, enhancement hatchery managers would release infected smolts without treatment under the supposition that the pathogen would die when exposed to salt water). The problem generally is manifested when infected smolts are transferred to seawater pens from fresh water. Formalin treatment is difficult and risky in seawater and treatment is best done while the fish are still in the hatchery.

*e) Rosette Agent ("Perkinsus").* This free-living choanoflagellate has caused epizootics typified by severe anemia in marine cultured salmonids in Washington (state) and Atlantic Canada. There are no known treatments.

*f) Myxosporeans.* There are hundreds of species, and they are not generally pathogenic except with heavy infestations. *Parvicapsula* sp. infects the kidneys, and can cause severe damage, while *Kudoa* sp. infects the flesh, lowering the market value. *Kudoa* has recently emerged as a significant problem in some regions (Conley 1994). *Myxobolus aeglefini* infect cartilage, and *Chloromyxum truttae* infect the liver and gall bladder. Control with drugs is limited.

*g) Microsporidians. Loma* (ex - *Pleistophora*) *salmonae* causes severe gill infections, can be carried from fresh water to seawater, and is directly transmissible in seawater. *Enterocytozoon salmonis* is associated with a marine anemia. Both of these diseases have been successfully treated orally with fumagillin DCH.

### *The Cost of Production*

In 1994, world farmed salmon production exceeded 450,000 t, and for the first time in history, exceeded the production of U.S. wild salmon. Norway alone could be producing 950,000 t in another decade, and within that time span is expected to increase exports to Japan (where farmed Atlantic salmon is now well accepted) from 24,000 t to 100,000 t (Johnson 1995). These are phenomenal figures and can only mean two things: (1) the cost of farming salmon is dropping dramatically; and (2) the salmon farming industry, which is now the domain of large conglomerates, is going to focus on aggressive global marketing (*see* Chapter 15).

In the early years of salmon farming, mortalities were high and feed conversions were poor. Even a dead salmon has consumed its share of rations while growing and before expiring, and contributes to the cost of feed. In the first ten years of salmon farming, feed costs usually amounted to 50-60% of production. In 1989, the Canadian government estimated that it cost (U.S.) $5.98/kg of chinook salmon production, and that feed costs were about $2.51/kg of harvested fish and may have been as high as 47% of all production costs. Norwegian production costs for Atlantic salmon between 1980-88 were estimated to be (U.S.) $4.41 to $5.88/kg (based on an average of 6.8NKr/$ during that period), and it was estimated that if those costs could be reduced by 20%, there would never be a problem with maintaining profitability.

Table 2 is a summary of the latest production costs for three of the larger producers of farmed Atlantic salmon in the world, Norway, Chile and Canada. Feed costs in Canada now average about $1.50/kg, $1.00/kg less than six years ago, and now down to 30% of production costs. Norway and Chile are even lower than 30%, and their total costs are at levels far below 1989 costs when inflationary factors are considered. Canada currently has a comparative disadvantage in the cost of smolts, but this is offset by the advantage of lower transportation costs. Canada should be able to increase smolt production efficiency and reduce the cost of smolts to the same level as in Norway and Chile. Since transportation costs may remain level, this would mean that Canada could produce farmed salmon at less delivered cost than most other countries. Note also that the cost of labour to produce fish on the farm is now less than processing them for market.

In Puget Sound (Washington state), some of the salmon farmers can look directly across the sound in one direction and see the processing plants where they take their fish for processing and shipping. They can look in another direction and see the jet planes taking off from Seattle's (major) International Airport, carrying their finished products to the far corners of the country and the globe. Some of these farms guarantee delivery to any major airport in the U.S. within 24 hours from the pens. Unfortunately, permits for salmon farms are now so restrictive that it is doubtful that production in Puget Sound can expand at the rate that we see in other regions in the world.

As production efficiencies continue to improve through breeding faster growing fish, increasing survival even more, developing more economical feeds, and improving mechanical equipment, costs will come down each year. The question is, can marketing develop consumer demand to keep pace with production?

**TABLE 2**

A summary of the average costs of farmed Atlantic salmon production in Chile, Norway, and Canada for 1994-95.

| | Average costs (and range)[a] in $US/kg (dressed weight) | | | | Average costs[a] expressed as a percentage (%) of the total cost/kg of dressed weight of the final product | | | |
|---|---|---|---|---|---|---|---|---|
| | Chile | Norway | Canada west coast | Canada east coast | Chile | Norway | Canada west coast | Canada east coast |
| Smolts | 0.44(±0.11) | 0.43(±0.05) | 0.77(±0.08) | 1.95(±0.08) | 9.13 | 8.78 | 15.95 | 18.42 |
| Feed | 1.32(±0.11) | 1.35(±0.08) | 1.49(±0.16) | 1.54(±0.11) | 27.40 | 27.70 | 30.75 | 29.98 |
| Labour | 0.23(±0.03) | .045(±0.03) | 0.43(±0.08) | 0.66(±0.11) | 4.79 | 9.23 | 8.88 | 12.85 |
| Vaccines/ medication/ health | 0.07(±0.02) | 0.04(±0.02) | 0.09(±0.02) | 0.09(±0.02) | 1.37 | 0.90 | 1.82 | 1.71 |
| Crop insurance | 0.08(±0.03) | 0.08(±0.08) | 0.09(±0.04) | 0.09(±0.04) | 1.60 | 1.58 | 1.82 | 1.71 |
| Other | 0.15(±0.02) | 0.09(±0.04) | 0.17(±0.05) | 0.10(±0.05) | 3.20 | 1.80 | 3.42 | 1.93 |
| Operations/ overhead | 0.40(±0.05) | 0.30(±0.05) | 0.33(±0.04) | 0.37(±0.06) | 8.22 | 6.08 | 6.83 | 7.28 |
| Depreciation | .023(±0.05) | 0.17(±0.05) | 0.09(±0.05) | 0.04(±0.02) | 4.79 | 3.38 | 1.82 | 0.86 |
| Financing/ interest charges | 0.04(±0.02) | 0.20(±0.09) | 0.10(±0.03) | 0.11(±0.04) | 0.91 | 4.05 | 2.05 | 2.14 |

TABLE 2 cont'd.

| | Average costs (and range)[a] in $US/kg (dressed weight) | | | | Average costs[a] expressed as a percentage (%) of the total cost/kg of dressed weight of the final product | | | |
|---|---|---|---|---|---|---|---|---|
| | Chile | Norway | Canada west coast | Canada east coast | Chile | Norway | Canada west coast | Canada east coast |
| Processing/ boxing | 0.39(±0.05) | 0.56(±0.05) | 0.59(±0.05) | 0.61(±0.05) | 7.99 | 11.49 | 12.30 | 11.78 |
| Sales/ marketing | 0.37(±0.02) | 0.35(±0.05) | 0.33(±0.02) | 0.31(±0.04) | 7.76 | 7.21 | 6.83 | 6.00 |
| Transportation to major markets | 1.10(±0.11) | 0.87(±0.05) | 0.36(±0.03) | 0.28(±0.05) | 22.83 | 17.79 | 7.52 | 5.35 |
| **Total cost F.O.B. major N. American markets** | **4.82(±0.64)** | **4.88(±0.61)** | **4.83(±0.65)** | **5.14(±0.67)** | | | | |

[a] $1.00 $US = $1.39 $CAN

Source: Adapted from Johnson (1995).

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## Chapter 10

# NUTRITION OF SALMONIDS

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## INTRODUCTION

Salmonids, like other animals, eat first to satisfy their energy requirements. Fish diets must include an adequate energy source plus sufficient indispensable amino acids, essential fatty acids, specific vitamins, and minerals to support life and promote adequate growth. Salmonids are poikilothermic and their metabolic rate is determined by the water temperature. Thus, the metabolic demand for maintenance of body temperature is minimized and less energy is used for this function. Salmonids have the additional advantage of living in a water environment where their streamlined bodies result in less energy expenditure for locomotion and nitrogenous wastes can be readily excreted as ammonia into the water mass. Hence, they are more efficient than land animals in both energy and protein utilization (Smith 1989).

The first well organized work on nutritional requirements was conducted at the Cortland Laboratory in New York as a joint venture between Cornell University, the New York Conservation Department, and the U.S. Fish and Wildlife Service (McCay and Tunison 1934). Gut content analysis for gross nutrients was used as a framework for artificial trout diets. Since salmonids can and often do use protein and lipid as their major energy sources, these original experiments led to erroneous conclusions on protein, carbohydrate, and fat levels for trout diets. Research efforts at the University of Wisconsin were focused on developing test diets that could be used to measure diet requirements (McLaren et al. 1947). The anti-anemic factor H (McCay 1927) was later demonstrated to be a combination of folic acid and vitamin $B_{12}$ (Halver 1969). Wolf (1951) developed a vitamin test diet for rainbow trout (*Oncorhynchus mykiss*) that could be used to qualitate most of the water-soluble vitamins for this species. An improved vitamin test diet, developed in 1950, contained purified protein, carbohydrate, lipid, and crystalline vitamin and mineral sources, and would rear Pacific salmon as well as rainbow trout through at least one reproductive cycle without the appearance of a specific vitamin deficiency syndrome (Halver 1953). Modifications of this diet were used to identify 11 water-soluble vitamin requirements and three of the four fat-soluble requirements for salmonids. Shortly thereafter, protein test diets were formulated and tentative protein requirements for small fish in the freshwater environment were catalogued (Halver et al. 1958). An amino acid test diet enabled classification of the 10 indispensable amino acids for chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), sockeye (*O. nerka*) salmon and rainbow trout (Halver 1957; DeLong et al. 1958; Halver and Shanks 1960). Feeding [$^{14}$C]-glucose and subsequently identifying labeled amino acids in the tissues of fish fed these diets showed that the same 10 indispensable amino acids were required by every salmonid tested.

A test diet for the lipid components enabled requirements for total lipid and essential unsaturated fatty acids to be catalogued for salmon and trout (Lee and Sinnhuber 1972). Mineral requirement studies were hampered by lack of experimental control over the inorganic components in the environment. Thus, only a few of these elements have been classified as dietary essentials and the dietary requirements may vary with the specific ingredients used and types of waters in which the fishes are reared (Lall 1989). Carbohydrate components have not been as thoroughly investigated as other gross nutrients but some work has shown salmonids can use reasonable amounts of carbohydrates to spare protein and lipid components of the diet (Buhler and Halver 1961).

A history of the first 50 years of work in fish nutrition was recently published (Rumsey 1994). A bulletin on requirements for these nutrients are summarized by NRC/NAS (1993) and more details can be found in Halver (1989*a*).

## BIOENERGETICS

Energy is released from dietary ingredients during metabolic oxidation of carbohydrates, proteins, and lipids. The energy requirements of an animal can be quantified by measuring the oxygen consumed or the heat produced in the reactions. Dietary gross energy is partitioned between digestible energy (DE), metabolizable energy (ME), net energy (NE), and recovered energy in final growth products (Smith 1989). Losses occur in fecal energy, urinary and gill excretions, and as heat during the chemical reactions (*see* Chapter 2). A constant supply of energy is necessary for all animals to maintain life. The energy for these systems comes from the feed consumed or, in times of starvation, from body stores. This energy is obtained primarily by oxidizing chemical bonds of complex molecules to a lower state with oxygen from the water. A review of nutritional energetics in fish is included in Fish Nutrition, 2nd Edition (Halver 1989*a*). Digestible energy is the difference between intake energy and energy lost in the feces.

Metabolizable energy can be calculated from the intake energy minus the fecal energy, the urinary energy and the gill energy losses. The recovered energy in growth and tissue components is the difference between the metabolizable energy and the maintenance heat energy used by the animal. The magnitude of each fraction is dependent on the quantity of the energy intake and the ability of the fish to digest and utilize that energy component. The level of feeding also increases the magnitude of some of these components. At a feeding level less than the maintenance level, weight loss occurs, after which more feed results in linear increases in DE, ME, and NE for production (Figure 1).

The oxidation of one mole of glucose to carbon dioxide and water releases about 700 kilocalories (kcal) of energy, which is trapped in high-energy adenosine triphosphate (ATP) bonds. The hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate releases energy that drives the energy-required reactions of metabolism. Theoretically, one mole of glucose should produce 85 moles of ATP but under physiological conditions only 39 moles of ATP are produced, giving a biological efficiency of about 40%. The remainder of the energy appears as heat, which in fish is rapidly lost to the water in which they are living.

Energy use in fish is more efficient than in homeothermic farm animals. Rainbow trout fed a balanced feed require not more than 1.5 kg of feed per kg of gain. Some well balanced diets have a dietary efficiency of one. The table of dietary protein and energy requirements per unit gain for salmonids and farm animals shows trout need about 8-10 kcal of digestible energy per gram of protein (Table 1). The fasting heat production of rainbow trout at Standard Environmental Temperature (SET) of 15°C requires only about 5% of the energy for maintenance required by a mammal or bird of the same size in a thermoneutral environment. This fasting heat production of a 100-g rainbow trout was about 1 kcal/d, according to Smith (1976). When the feed contains 3 kcal of DE per gram, and trout have an intake of 6 kcal of digestible energy per day, about six times the maintenance requirement is available for other work. Thus, about 85% of the digestible energy of the feed is theoretically available for growth.

Many factors affect energy requirements. Demands for maintenance and physical activity must be satisfied before any energy is available for growth. When fish are forced to swim actively, energy will be wasted. Any external disturbance will waste energy. Stress and disease can reduce growth. Smith (1989) estimated that voluntary activity uses about twice as much energy as basal metabolism. The energy expended for physical activity will appear as heat and will be absorbed by the surrounding water.

As fish are poikilothermic, temperature has the greatest effect of any of the factors on energy requirements. A comparison of the effect of temperature on heat production in fish and mammals shows that as body temperature increases in fish (environmental temperature), the heat production also increases (Figure 2).

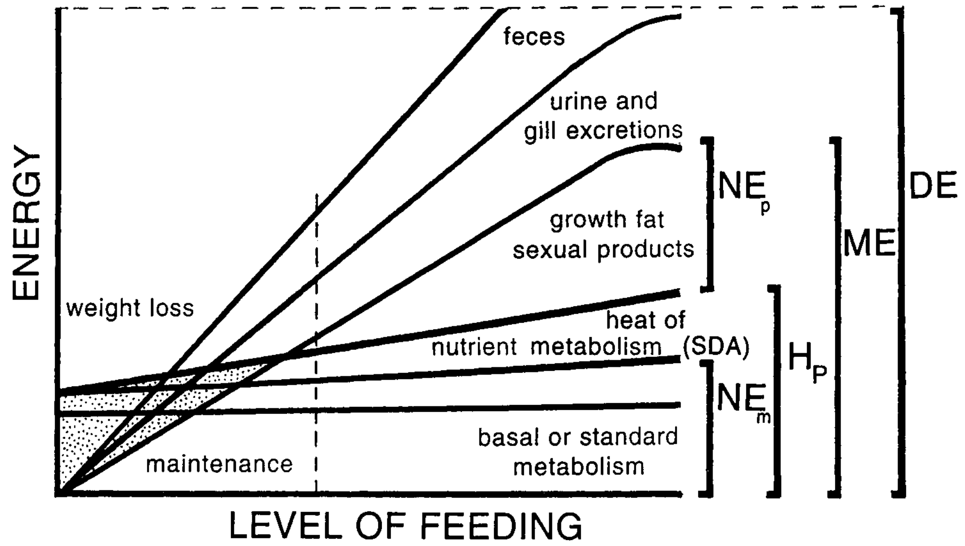

FIGURE 1. Energy distribution in growing fish at different levels of feeding (from NRC/NAS 1981). (DE - digestible energy, ME - metabolizable energy, $NE_P$ - net energy for production, $NE_M$ - net energy for maintenance, $H_P$ - heat production)

Food passage through the gut may alter with time, feeding frequency, other activity, or water flow. When the diet is accepted at near normal feeding rates, then digestible energy can be estimated using an inert marker incorporated into the feed and the following formula:

$$\text{Digestibility} = 1 - \frac{\%\ \text{indicator in feed}}{\%\ \text{indicator in feces}} \times \frac{\%\ \text{nutrient in feces}}{\%\ \text{nutrient in feed}} \tag{1}$$

This approach assumes that: (1) fecal matter collected is representative of that which would be voided naturally; (2) loss by leaching is not significant; (3) marker is not absorbed or retained by test animal; (4) marker does not interfere with the digestion and absorption of nutrients; and (5) fecal sample is not contaminated with uneaten food. A direct method for measuring not only DE but ME as well has been described by Smith (1989) and by Cowey and Walton (1989).

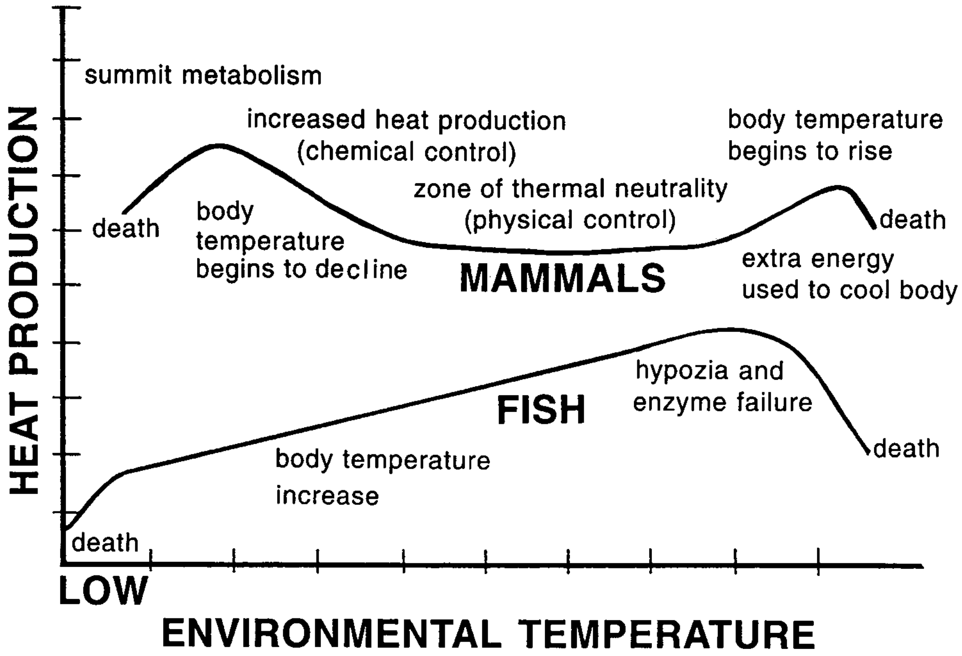

FIGURE 2. Heat production in fish and mammals relative to environmental temperature (from Smith 1989).

## VITAMINS

The water-soluble vitamins include eight members of the vitamin B complex plus the water-soluble essential nutritional factors choline, inositol, and ascorbic acid. Thiamin was used to cure the Chastex-type paralysis of diet disease in rainbow trout by Schneberger (1941). This was the first demonstration of a specific role for a water-soluble vitamin in alleviating a deficiency disease. A more complete description of the vitamins and diagrams of their chemical structures can be found in Halver (1989*b*) and NRC/NAS (1993) but are also summarized as follows.

### *Thiamin*

Thiamin hydrochloride is a water-soluble, colourless, monoclinic crystalline compound which is comparatively stable to dry heat but is rapidly broken down in neutral or alkaline solutions and can be split by sulfites into pyrimidine and thiazole moieties. Thiamin is part of the enzyme cocarboxylase, which participates in the oxidative decarboxylation of $\alpha$-keto acids, releasing carbon dioxide. Thiamin is an antagonist to

TABLE 1

Dietary energy to protein ratios in selected fishes and domestic animals[a].

| Species | Feed/gain | kcal energy/kg gain | kcal DE/g protein |
|---|---|---|---|
| Rainbow trout | 1.0 - 1.5 | 3500 - 4500 | 8 - 10 |
| Channel catfish | 1.2 - 1.8 | 3000 - 3500 | 10 - 12 |
| Chicken (broiler) | 2.5 - 3.0 | 6000 - 7500 | 13 - 15 |
| Swine | 3.5 - 4.0 | 12000 - 13000 | 19 - 20 |
| Cattle (beef) | 7.0 - 8.0 | 18000 - 20000 | 25 |

[a] calculated from NRC Tables (Smith 1989)

acetylcholine and stands at the crossroads of carbohydrate intermediary metabolism as the endpoint in the anerobic process before decarboxylation and oxidative reactions begin the reverse process. An interrelationship between thiamin and lipoic acid occurs in other animals. Brin (1963) used the role of thiamin pyrophosphate in the transketolase systems as a method for estimating the thiamin status in salmon and trout. Morieto et al. (1986) also used this technique to correlate thiamin intake with the physiological state of trout. Thiamin deficiency progresses from anorexia to neurological signs of susceptibility to shock or to spastic convulsions, instability and loss of equilibrium. Edema is a common manifestation of thiamin deficiency. Vitamin deficiency syndrome for thiamin compared to the other water-soluble vitamins is listed in Table 2.

Storage levels of the vitamin in the liver have been measured and differences in growth response and food conversion calculated. One series of experiments were conducted by assaying meat-meal mixtures for thiamin content, feeding these rations to groups of young fish until positive growth differences were observed, and then assaying the livers of the fish for the vitamin content. The minimum dietary intake which resulted in maximum liver thiamin content was selected as that diet treatment that would satisfy the vitamin requirement (Phillips et al. 1946). A typical Almquist plot of thiamin intake and liver storage levels is shown in Figure 3. This approach is the most conservative method to estimate the thiamin requirement. When erythrocyte transketolase activity is used, maximum activity is obtained at lower levels of thiamin intake. When only growth response criteria are used, even lower levels of thiamin are calculated for maximum growth response. Therefore, the requirement for this vitamin and for many of the other water-soluble vitamins can be measured with different criteria, as illustrated in Figure 4. As these water-soluble vitamins are integrated into essential metabolic processes, the fish nutritionist must decide what level of that particular vitamin should be present in the feed intake of the fish reared. These chemicals are stored for varying lengths of time, are essential for life functions, and may have increased demand under certain stress or

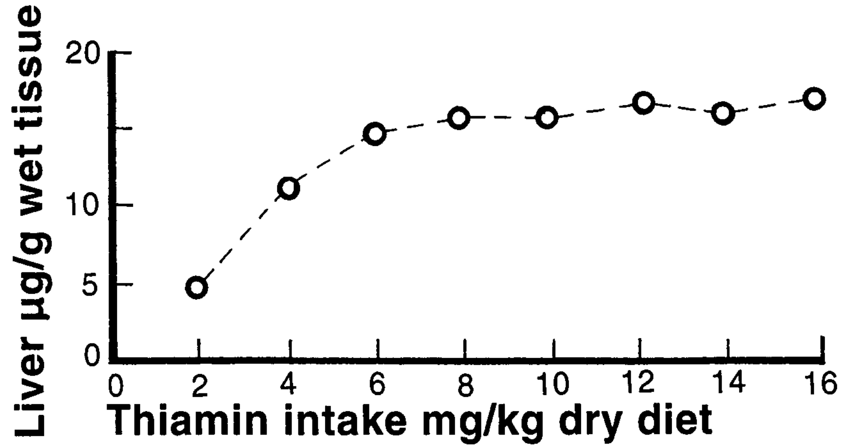

FIGURE 3. Thiamin intake and storage (from Halver 1989*a*).

handling conditions. Thus, the levels recommended are insurance policies for healthy fish with maximum growth potential in specific systems used. The dietary requirement for young Pacific salmon reared on chemically defined diets is estimated at 10-15 mg/kg dry diet for maximum liver storage levels. Rainbow, brook (*Salvelinus fontinalis*), and brown (*Salmo trutta*) trout require about the same levels for maximum liver storage but only about 1-2 mg/kg dry diet for maximum growth response. Table 3 lists trout and salmon requirement estimates using maximum liver storage levels, clinical assay, and maximum growth as determinant criteria.

### *Riboflavin*

Riboflavin is a yellow-brown crystalline pigment, slightly soluble in water with yellow-green fluorescence. It is stable to oxidizing agents and in dry form is heat stable. It is decomposed on irradiation or visible light, breaking down to lumiflavin. Riboflavin acts as coenzyme flavin adenine dinucleotide or flavin mononucleotide. The flavoproteins function in enzymes in tissue respiration and are coupled in hydrogen transport to catalyze the oxidation of reduced-pyridine nucleotides niacin adenine dinucleotide (NADH) and niacin adenine dinucleotide phosphate (NADPH). These co-enzymes function in many oxidation and reduction reactions. Riboflavin is also involved in the retinal pigment during light adaptation and lack of it causes impaired vision and photophobia in fish (Halver 1989*b*).

A summary of riboflavin deficiency signs in fish is listed in Table 2. Tissue storage is exhausted in young salmonids after 10-12 weeks on riboflavin deficient diets. Poor

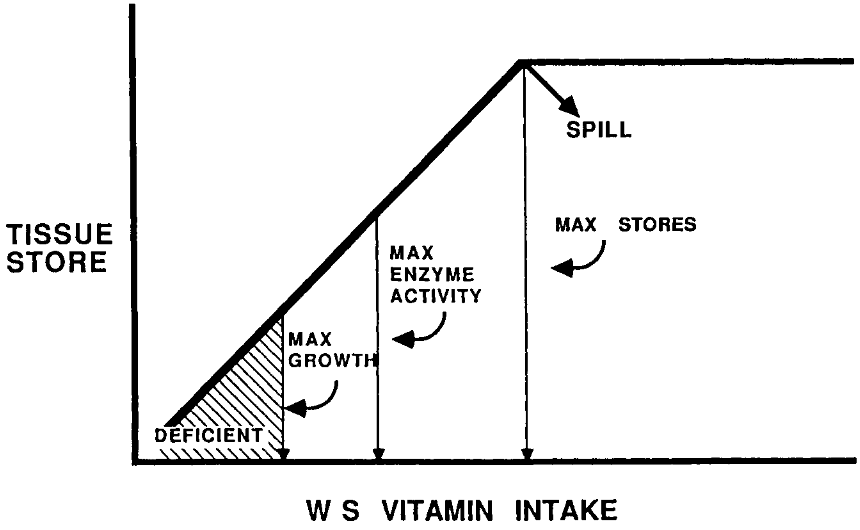

FIGURE 4. Water-soluble (WS) vitamin intake and tissue stores (from EIFAC 1994).

appetite and poor diet efficiency are the first signs, followed by photophobia, then cataracts, some corneal vascularization, ocular haemorrhage, incoordination, and general anemia. Abnormal pigmentation of skin and iris has been noted. Replacement of riboflavin in the diet reduces the symptoms except when cataracts have developed. This irreversible condition will continue in monolateral cataract-affected fish whereas bilateral cataract-affected fish generally succumb to starvation and death (Halver 1989*b*). The requirements of salmonids for riboflavin under laboratory controlled experimental conditions in 10-15°C water are listed in Table 3.

Values for trout are somewhat lower than for salmon, which were fed test diets containing increments of crystalline riboflavin. During treatment, the growth response was measured for 10 weeks and then the livers assayed to determine which diet treatment would induce maximum liver storage. Requirements may vary depending on balance of other dietary ingredients and environmental conditions under which the fish are reared. The requirements listed in Table 3 are conservative tentative requirements that should satisfy biological needs for normal growth, health, and physiological functions. These requirements are for very young fish with the assumption that the vitamin requirement of small fish will be greater than that of larger fish, which may develop metabolic enzyme systems with the ability to synthesize or spare at least some of the vitamins.

**TABLE 2**

Signs of vitamin deficiency syndromes in salmonids.

| Vitamin | Deficiency signs |
|---|---|
| Thiamin | Nervous disorders, loss of equilibrium, hyperirritability, convulsions, low transketolase activity in erythrocytes and kidney |
| Riboflavin | Lethargy, dark pigmentation, spinal deformities, fin haemorrhage and erosion, photophobia, corneal vascularization, eye haemorrhage, reduced activity of erythrocyte, glutathione reductase |
| Pyridoxine | Nervous disorders, anemia, eleptiform convulsions, hyperirritability, erratic spiral swimming, low resistance to handling |
| Pantothenic acid | Clubbed gills, distended operculum, atrophied pancreatic acinar cells, mortality |
| Niacin | Skin, fin, and colon lesions, photosensitivity, sunburn, abdominal edema, muscular weakness, anemia |
| Biotin | Degenerative gill lamellae, skin lesions, muscle atrophy, spastic convulsion, reduced hepatic acetyl CoA, carboxylase, and pyruvate carboxylase, lipid infiltration of liver, degeneration of pancreatic acinar cells |
| Folate | Lethargy, slow growth, dark skin colouration, anemia |
| Vitamin $B_{12}$ | Microcytic hypochromic anemia, fragmented erythrocytes |
| Choline | Fatty liver, exophthalmia, extended abdomen, haemorrhagic kidney and intestine |
| Inositol | Dark skin colouration, distended abdomen, anemia, fin erosion, reduced activity of cholinesterase and transaminase |
| Ascorbic acid | Lethargy, petechial haemorrhages, exophthalmia, intramuscular haemorrhage, distorted gill filaments, opercles, lordosis, scoliosis, ascites, anemia, thin cranial wall |

TABLE 2 cont'd.

| Vitamin | Deficiency signs |
|---|---|
| Vitamin A | Skin depigmentation, exophthalmia, eyes and lens displacement, corneal thinning, retinal degeneration, edema, ascites |
| Vitamin D | Impaired calcium homeostasis, tetany of skeletal muscle, increased liver lipid |
| Vitamin E | Skin depigmentation, anemia, ascites, abnormal erythrocytes, muscular dystrophy, edema |
| Vitamin K | Prolonged blood clotting, anemia, haemorrhagic gills and eyes |

### *Pyridoxine*

Pyridoxine hydrochloride is readily soluble in water and is heat stable in either acid or alkaline solutions. The so-called vitamers $B_6$ consist of pyridoxine, pyridoxal, pyridoxamine, and other derivatives that have biological activity, or can be converted into the biologically active form pyridoxal phosphate. Pyridoxal phosphate acts as the coenzyme for decarboxylation of amino acids. It is also involved in the aminotransferase enzymes and is required for the synthesis of the neurotransmitters 5-hydroxy-tryptamine and serotonin from tryptophan. Therefore pyridoxine deficiencies listed in Table 2 include nervous disorders, hyperirritability, convulsions, and premortum rigor.

### *Pantothenic Acid*

Pantothenic acid is a yellow viscous oil and therefore the compound generally used in fish nutrition is the calcium salt. This salt is stable to oxidation reduction agents and is labile to dry heat. The alcohol derivative is also active but the optical isomer is reported to be physiologically inert. Pantothenic acid is part of acetyl-coenzyme A, which occurs in many enzymatic processes involving 2-carbon compounds. The 2-carbon fragment, or acetyl-coenzyme A, is an essential intermediate in metabolism involved in most acylation reactions in the body. It is also involved in adrenal (interrenal) functions and in the production of cholesterol. Coenzyme A can be found in many steps of intermediary metabolism of carbohydrates, fats, and proteins. Thus, pantothenic acid is a key nutrient for normal physiology.

TABLE 3

Vitamin requirements of salmonids[a]. Requirement units are in mg/kg dry weight unless noted otherwise.

| Vitamin | Species | Requirement | Criteria[b] | Reference |
|---|---|---|---|---|
| Thiamin | Pacific salmon | 10-15 | MLS | Halver 1972 |
| | Rainbow trout | 1-10 | WG, ADS | McLaren et al. 1947 |
| Riboflavin | Pacific salmon | 20-25 | MLS | Halver 1972 |
| | | 7 | WG, ADS | Leith et al. 1990 |
| | Rainbow trout | 5-15 | WG, ADS | McLaren et al. 1947 |
| | | 6 | MLS | Takeuchi et al. 1980 |
| | | 3 | Enz | Hughes et al. 1981 |
| | | 2.7 | MLS, Enz | Amezaga and Knox 1990 |
| Pyridoxine | Pacific salmon | 10-15 | MLS | Halver 1972 |
| | | 6 | MG | Leith et al. 1990 |
| | Atlantic salmon | 5 | MG | Lall and Weerakoon 1990 |
| | Rainbow trout | 2 | MG | Woodward 1990 |
| | | 3-6 | Enz | Woodward 1990 |
| Pantothenic acid | Pacific salmon | 40-50 | MLS | Halver 1972 |
| | | 17 | MG | Leith et al. 1990 |
| | Rainbow trout | 20 | MG | Cho and Woodward 1990 |
| Niacin | Pacific salmon | 150-200 | MLS | Halver 1972 |
| | Rainbow trout | 10 | MG | Poston and Wolfe 1985 |
| Biotin | Pacific salmon | 1-1.5 | MLS | Halver 1972 |
| | Rainbow trout | 0.08 | MG | Woodward and Frigg 1989 |
| | | 0.14 | Enz | Woodward and Frigg 1989 |
| | Lake trout | 0.1 | MG | Poston 1976*b* |
| | | 0.5-1.0 | Stamina | Poston 1976*b* |
| Folic acid | Pacific salmon | 6-10 | MLS | Halver 1972 |
| | | 2 | MG | Leith et al. 1990 |
| | Rainbow trout | 1.0 | MG | Cowey and Woodward 1993 |
| $B_{12}$ | Pacific salmon | 0.015-0.02 | MLS | Halver 1972 |

**TABLE 3 cont'd.**

| Vitamin | Species | Requirement | Criteria[b] | Reference |
|---|---|---|---|---|
| $B_{12}$ cont'd. | Rainbow trout | R | | Phillips et al. 1964 |
| Choline | Pacific salmon | 600-800 | MLS | Halver 1972 |
| | Rainbow trout | 50-100 | MG | McLaren et al. 1947 |
| | | 700-800 | Chem | Rumsey 1991 |
| | | 1000 | MG | Ketola 1976 |
| Inositol | Pacific salmon | 300-400 | MLS | Halver 1972 |
| | Rainbow trout | 250-500 | MG | McLaren et al. 1947 |
| Ascorbic acid | Pacific salmon | 50 | MLS | Halver 1969 |
| | Atlantic salmon | 50 | MLS | Lall et al. 1990 |
| | Rainbow trout | 100 | MLS | Halver et al. 1969 |
| | | 40 | MG | Hilton et al. 1978 |
| Vitamin A | Pacific salmon | R | MG | Halver 1972 |
| | Rainbow trout | 2500 IU | MG | Kitamura et al. 1967 |
| Vitamin D | Pacific salmon | NR | MG | Halver 1972 |
| | Rainbow trout | 1600-2400 IU | MG | Barnett et al. 1982 |
| Vitamin E | Pacific salmon | 40-50 mg | MLS | Halver 1972 |
| | | 30 | MG | Woodall et al. 1964 |
| | Atlantic salmon | 35 | MG | Lall et al. 1988 |
| | Rainbow trout | 30 | MG | Woodall et al. 1964 |
| | | 25 | MG | Hung et al. 1980 |
| | | 100 | MLS | Watanabe et al. 1981 |
| | | 50 | Enz | Cowey et al. 1983 |
| Vitamin K | Pacific salmon | R | MG | Halver 1972 |
| | Lake trout | 0.5-1.0 | Chem | Poston 1976*a* |

[a] adapted from Halver (1989*b*) and NRC/NAS (1993)

[b] Abbreviations:

| | |
|---|---|
| ADS = | Absence of deficiency signs |
| Chem = | Chemical analysis |
| Enz = | Clinical enzymology |
| MG = | Maximum growth |
| MLS = | Maximum liver storage |
| NR = | Requirement has not been demonstrated |
| R = | Required |
| Stamina = | Swimming stamina test |
| WG = | Weight gain |

Pantothenic acid deficiency signs are listed in Table 2, with typical hyperplasia of gill tissues and subsequent necrosis being the most characteristic. The deficiency signs are reversible when pantothenic acid is added to the diet; however, some evidence of necrosis and scarring are still present as the gills, filaments, and lamellae repair. Estimates for growth requirements are shown in Table 3.

### *Niacin*

Niacin or nicotinic acid amide is present as NAD and NADP for the removal of hydrogen ions from substrates in the transfer of hydrogen or electrons to another coenzyme in hydrogen transport systems. These enzymes alter between the oxidized and reduced state of the coenzymes NAD-NADH and NADP-NADPH, which are anaerobic reactions. The second type of oxidation-reduction reaction is coupled to electron transport when oxidation of reduced NADH or NADPH occurs in aerobic reactions. In respiration, these compounds are involved in synthesis of high energy phosphate bonds, ATP and ADP, which furnish the energy for certain steps in glycolysis, in pyruvate metabolism, and in photosynthesis. Niacin is also involved in lipid metabolism, amino acid protein metabolism, and photosynthesis. Thiamin and niacin are both involved in coenzyme systems of carbohydrate metabolism, where ingested material is oxidized to heat and generates high energy phosphate bonds for subsequent physiological reactions.

Niacin deficiency syndrome in fish is less specific than for some of the other water-soluble vitamins. Muscle spasms occasionally occur when fish are apparently resting. A predisposition to sunburn in trout confined in open shallow waters has been described (DeLong et al. 1958*a*). Muscular weakness was also noted, and a general lack of appetite was the first symptom observed (Table 2).

Estimated requirements for niacin in young fish are listed in Table 3. Rainbow trout and salmon requirements were determined by feeding test diets containing different increments of niacin, and then measuring growth and point of maximum liver storage at the end of the experimental period. The salmon requirements appeared to be about twice that of trout. Deficiency symptoms were reduced by replacement of niacin in the diet. Dermal lesions occurred after 2 weeks exposure of rainbow trout to ultraviolet radiation, with a total loss of mucus-producing cells in the area of the lesion. Very high intake of niacin (10,000 mg/kg dry diet) increased liver fat and tended to reduce growth rate in small brook trout (Poston 1969). This was the first reported incidence of hypervitaminosis in the water-soluble vitamins, but about 1,000 times the normal growth requirement was needed to induce this toxic effect. Several antimetabolites to niacin occur, including thioacetamide and phenothiazine (Halver 1989*b*).

### *Biotin*

Biotin is a monocarboxylic acid, slightly soluble in water and alcohol, and is easily destroyed by acid, alkalis, and oxidizing agents. It can be easily bound to avidin, a protein found in raw egg white, which makes it unavailable to fish. Biotin is required in several specific carboxylation and decarboxylation reactions. It is involved in fixing carbon dioxide and as a coenzyme for proprionyl coenzyme A in formation of proprionic acid. It is involved in purine synthesis and the coenzyme of malonyl coenzyme A involved in the elongation of fatty acids. It is involved in general lipid synthesis and in deamination and biosynthesis of citrulline. Biotin is also involved in the conversion of saturated fatty acids to the stable *cis* form, which is essential for the structural activity of biologically active fatty acids in lipoproteins and membranes. Biotin deficiency signs in salmonids are skin disorders, muscle atrophy, and lesions in the colon (Table 2). Brook trout have been reported to develop a blue slime patch disease from biotin deficiency, and quantitative studies indicate brown trout may require more biotin than brook or rainbow trout. Biotin requirements are included in Table 3. Clinical assessment of biotin status has been suggested by measurements of the liver pyruvate carboxylase activity (Walton et al. 1984).

### *Folic Acid*

Folic acid is required for normal blood cell formation and is involved as a coenzyme in one carbon transfer mechanisms. Folic acid is transformed into 5-formidal-5-6-7-8-tetrahydro-pteroyl-glutamic acid, which is the active compound in one-carbon metabolism systems such as serine and glycine intraconversion, from methionine to homocystine synthesis, histidine synthesis, and pyrimidine synthesis for basis of the nucleotides DNA and RNA. Folic acid is also involved in the conversion of proforms of erythrocytes into mature erythrocytes in salmonids.

A deficiency sign is macrocytic normochromic anemia, which can be readily reversed by the addition of folic acid to the diet. An anterior kidney imprint will disclose normal erythropoeisis or the presence of many senile cells in folic acid deficiency. Gross hematology is a simple clinical method for the assessment of folic acid status of the salmonid (Tables 2 and 3) (Halver 1989*b*).

### *Vitamin $B_{12}$*

Vitamin $B_{12}$, or cyanocobalomin, is involved with folic acid in hemopoiesis. Many structural isomers of vitamin $B_{12}$ have been described with varying degrees of $B_{12}$ activity. It is a large molecule with a cobalt atom in the central location in planar structure with a cyano group linked with the central cobalt. It is stable to mild heat but is rapidly destroyed by dilute acid or alkali.

The deficiency sign in fish is primarily anemia with many erythrocytes fragmented and in abherrant form (Table 2). It is difficult to assay for vitamin $B_{12}$ in diets, and the long induction period for anemia to develop has limited investigation in other species of salmonids.

The vitamin $B_{12}$ requirements for chinook salmon were determined by feeding chemically defined diets to young fish and assaying for maximum liver storage levels for the different increments of $B_{12}$ fed. When these levels of $B_{12}$ are found in diets for other salmonids, anemias do not appear and the $B_{12}$ requirement appears to be satisfied.

### *Ascorbic Acid*

Ascorbic acid, or vitamin C, was first suggested to be required by rainbow trout by McLaren et al. (1947). Twenty years later, Kitamura et al. (1965) showed a definite need for vitamin C and then four years later, L-ascorbic acid was demonstrated required by salmon (Halver et al. 1969). L-Ascorbic acid ($C_1$) acts as a biological reducing agent in hydrogen transport. It is involved in many enzyme systems for hydroxylation of proline, lysine, tryptophan, tyrosine, and is involved in detoxification of aromatic drugs through induction of mixed function oxidases (MFOs). It is also involved in hydroxylation of steroids and for hormone formation. One major primary function is in the conversion of procollagen into collagen for the synthesis of cell membrane structures. Scurvy in fish is similar to scurvy in other animals that cannot synthesize $C_1$. Deficiency signs include abnormal collagen formation, petechial haemorrhages, hyperplasia of gill support tissues, scoliosis, lordosis, thin cranial wall, and in advanced cases, anemia with abnormal erythrocyte cell formation (Table 2).

Requirements for vitamin $C_1$ vary with fish size, diets fed, stress, and husbandry conditions used (Table 3). Vitamin $C_1$ is very prone to oxidation and is rapidly lost during feed manufacture, storage, and even during feeding conditions. Estimates of requirements originally used crystalline L-ascorbic acid, and assays for levels present in the diet as fed and tissue assays used techniques that may not have disclosed levels of material actually available to the fish. Recent improved chemical assays using high pressure liquid chromatography techniques and more stabilized sources for ascorbic acid have indicated vitamin C requirements exist for growth, and higher requirements occur for conditions of stress, disease, or trauma (Halver et al. 1993). Original estimates of 100 mg/kg dry diet, using L-ascorbic acid as the ingredient, were reduced to 30 mg/kg dry diet when ascorbic-2-sulfate (C2S), or ascorbate-2-monophosphate (C2MP) were used as a C-equivalent source. Requirements of four to eight times the growth requirement may be necessary for tissue wound repair (Halver et al. 1969) or resistance to disease organisms such as *Vibrio anguillarum* (Navarre and Halver 1989). Therefore, the vitamin C requirement is reflected by the husbandry condition, the stress or disease challenge, and the size of the fish being reared.

Some species differences have been reported with rainbow trout apparently the most sensitive salmonid to vitamin C requirements, with other trout species, Atlantic salmon (*Salmo salar*), and chinook and coho salmon requiring about half the intake of rainbow trout for maximum growth. The ubiquitous nature of vitamin C in essential physiological functions indicates special care is needed to assure adequate intake of this sensitive water-soluble vitamin for maximum growth and fish health (Halver 1989*b*; NRC/NAS 1993).

### *Choline*

Choline has three major metabolic functions. Phosphatidal choline is a structural component in biological membranes, acetylcholine acts as a neural transmitter, and choline serves as a labile methyl group source for methylation reactions. Choline is a very strong organic base, is very hydroscopic, soluble in water, and is stable to heat and acid solutions, but it decomposes rapidly in alkaline solutions. It is a strong base for many chemical reactions in the body. The deficiency signs listed in Table 2 involve poor growth and poor food conversion. Choline reacts almost like an amino acid in the growth response, with very little growth occurring without choline and growth directly related to the choline content of the diet up to the required levels. The requirements are listed in Table 3 and generally reflect adequate choline intake for normal growth and fish production.

### *Inositol*

Inositol, or muscle sugar, exists as seven optically inactive and two optically active isomers of hexahydroxycyclohexane. One of the optically active forms, myoinositol, has biological activity. The main function of myoinositol is as a structural component in living tissues. It does have lipotropic activity preventing accumulation of cholesterol in one type of fatty liver disease, and is involved with choline for normal lipid metabolism. The deficiency signs for fish are generally poor growth with increased gastric emptying time. Some edema has been reported, and distended stomachs have been observed in salmon and trout. Table 2 lists the inositol-deficiency syndrome. Requirements of salmonids for inositol are listed in Table 3.

### *Vitamins A, D, E and K*

The fat-soluble vitamins A, D, E, and K are absorbed with fat materials in the gut and are stored in the neutral lipid of the tissues until needed. It is possible to produce hypervitaminosis in the fat-soluble vitamins if the intake exceeds dietary requirements, as these materials are slowly detoxified and eliminated from the system (Figure 5). Many isotels for these vitamins are well known, but only the major ones will be discussed in the

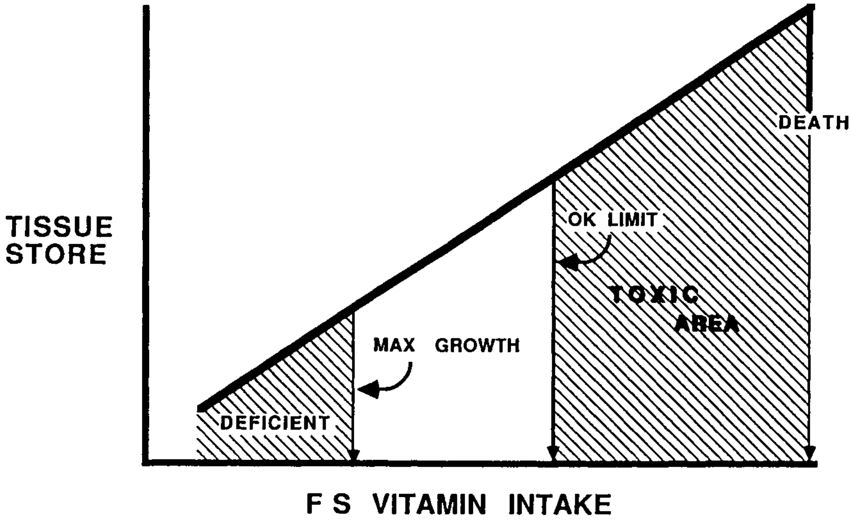

FIGURE 5. Fat-soluble (FS) vitamin intake and tissue stores (from EIFAC 1994).

following paragraphs. The chemistry and biochemistry of these compounds have been reviewed recently by Sargent et al. (1989).

*Vitamin A.* Vitamers A, retinol ($A_1$) or retinal ($A_2$), and retinoic acid all have vitamin A functions essential for maintaining epithelial cells. Hypovitaminosis A is characterized by poor growth, poor vision, haemorrhage in the eyes and base of fins, displaced lenses, degeneration of the retina, sometimes edema, body pigment changes, photophobia, and abnormal bone formation. Betacarotine can be used as a source of vitamin A at warmer water temperatures, but at very low temperatures of some salmonid environments it is poorly used as an A source.

An excess of vitamin A active compounds results in an enlargement of liver and spleen, skin lesions, epithelial keratinization, hyperplasia of head cartilage, and abnormal bone formation, including fusion of vertebrae. Deficiency can be alleviated by addition of a vitamin A source for the diet and hypervitaminosis can be recovered by reducing the A content of the diet. The requirements for salmonids tested are listed in Table 3.

*Vitamin D.* Vitamers D, cholecalciferol ($D_3$) or ergocalciferol ($D_2$), are hydroxylated in the liver to 25-hydroxy-D forms. 1-25-Dihydroxy vitamin $D_3$ is the biologically active form responsible for mobilization, transport, absorption, and use of calcium and phosphorus in muscle function and bone structure formation. Lovelace and Podoliak (1956) have shown that rainbow trout and other salmonids can efficiently sequester calcium from the water, and consequently have a low vitamin D requirement for calcium absorption mechanisms (Halver 1989*b*). Impaired calcium function and tetany in white muscle fibers can occur in D deficiency. Vitamin D requirements of fishes in normal waters with some calcium should be low. Work with chinook and coho salmon using purified diets devoid of vitamers D failed to demonstrate a D requirement when any appreciable calcium content was present in the water supply. Studies with rainbow trout in calcium-free waters have demonstrated a positive requirement for vitamin D however. Probably most commercial fish diets will have adequate amounts of vitamin D present to satisfy the low requirements for this vitamin for salmonids (Halver 1989*b*; NRC/NAS 1993).

*Vitamin E.* Vitamers E, or the tocopherols, are involved as inter- and intra-cellular antioxidants to maintain homeostasis of labile metabolites in tissue. As physiological antioxidants, they protect oxidizable vitamins and polyunsaturated fatty acids. Vitamin E ($\alpha$-tocopherol) functions with selenium and ascorbic acid in the enzymes glutathione peroxidase and superoxide dismutase to stop chain reactions of peroxidation. The deficiency signs are listed in Table 2 and also include muscular dystrophy, atrophy and necrosis of white muscle fibers, heart edema, and increased capillary permeability. Erythrocyte fragility tests have been used as an indicator of vitamin E status in fish. Peroxide hemolysis of the red blood cells has been used to determine E deficiency in rainbow trout. A better method is *in vitro* ascorbic acid-stimulated lipid peroxidation in liver microsomes of rainbow trout. The E requirement is also reflected in levels of polyunsaturated fatty acids present in the diet, and the requirement listed is for normal diet mixtures containing not more than 3% of the diet as polyunsaturated fatty acids. Hypervitaminosis E has been described and involves poor growth, toxic liver reactions, and eventually death (Halver 1989*b*; NRC/NAS 1993).

*Vitamin K.* Vitamers K, $K_1$, and other quinones with K activity are required for stimulation of prothrombin activity in plasma and synthesis of blood clotting factors. The deficiency signs include increased blood clotting time in salmon fed diets devoid of vitamers K. During prolonged deficiency states, anemia and haemorrhagic areas appear in the gills, eyes, and some vascular tissues. Intake of vitamers K at 2,000-3,000 mg/kg dry diet can be tolerated for short time periods by trout, but higher levels may cause liver toxicity and death.

### *Other Vitamin-like Factors*

Other vitamin-like or unknown factors exist that may influence osmoregulation and cell membrane permeability. These may be especially important for anadromous fish. Relationships between vitamers C, E, K, and A in respiration have been observed, but the exact role of these and other factors such as selenium, zinc, copper, and sodium chloride have not been defined. Both pituitary function and thyroid function are associated with smoltification, specifically with rapid development of gill secretory cells to maintain salt and water balance in the new environment (*see* Chapter 8). Rutin and esculin are citrovorum-like factors that may affect capillary permeability. Recently the role of certain polyunsaturated fatty acids in the cell membrane lipoprotein components in phospholipids has been described (Dey et al. 1993). Lecithin itself may be a growth-promoting factor above and beyond the choline content of this compound. Evidence is rapidly accumulating that a more exact balance of many chemical compounds involved in interrelationships with metabolic enzyme functions may explain the apparent effects of some unknown compounds in natural diet ingredient mixtures that are fed to fish. Salmonids, like other living animals, therefore require adequate intake of at least eleven of the water-soluble and four of the fat-soluble vitamins that have been demonstrated to be essential in other animals (Halver 1989*b*). For complete details of the structures, chemical characteristics and antimetabolites of all vitamins important in fish nutrition, see Halver (1989*b*).

## PROTEIN AND AMINO ACIDS

Proteins are made up of about 20 $\alpha$-amino acids linked into chains by peptide bonds. Salmonids do not have a requirement for protein *per se*, but rather for the various indispensable and dispensable amino acids that constitute the protein matrix. However, it is convenient to assume the protein is balanced for indispensable amino acid content, and hence convenient to estimate the gross balanced protein requirement of fishes. The estimated protein requirements of several species of juvenile salmonids are summarized in Table 4. These requirements have been estimated from dose-response curves with the minimum amount of dietary protein that resulted in maximum growth. One must first calculate that portion of the protein component that is digested and absorbed. Only if the amino acids are absorbed and contribute to tissue synthesis can protein be deposited for tissue repair and for growth. Protein and amino acid requirements have been reviewed by Wilson and Halver (1986).

Measurements of Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) can be used as estimates of the value of the protein in various diet mixtures. The PER equals the weight gain divided by the weight of protein fed and can be conveniently calculated from the weight gain in a group of fish fed the protein contained in a diet. Apparent NPU equals the weight of protein retained in the carcass divided by the weight of protein fed in feed. Assumptions used are: (1) 6.25 × nitrogen = % protein in feed; (2) feed fed is all consumed; (3) 6.25 × nitrogen = % protein in fish carcass; and (4) alternatively, fish carcass contains 21% protein.

### *Indispensable Amino Acids*

Every fish examined to date requires the same 10 indispensable amino acids for growth. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine,

TABLE 4

Estimated dietary protein requirements of salmonids as a percentage of the diet[a].

| Species | Size | Requirement | Reference |
|---|---|---|---|
| Chinook salmon | juvenile | 40 | DeLong et al. 1958 |
| Coho salmon | juvenile | 40 | Zeitoun et al. 1974 |
| Sockeye salmon | juvenile | 45 | Halver et al. 1964 |
| Atlantic salmon | juvenile | 45 | Lall and Bishop 1977 |
| Atlantic salmon | fry | 45-50 | Hardy 1991 |
| Atlantic salmon | juvenile | 40 | Hardy 1991 |
| Atlantic salmon | grow-out | 40 | Hardy 1991 |
| Atlantic salmon | broodstock | 45 | Hardy 1991 |
| Rainbow trout | juvenile | 40 | Satia 1974 |
| Rainbow trout | fry | 45-50 | Hardy 1991 |
| Rainbow trout | juvenile | 40 | Hardy 1991 |
| Rainbow trout | grow-out | 35 | Hardy 1991 |
| Rainbow trout | broodstock | 40 | Hardy 1991 |

[a] adapted from NRC/NAS (1993)

threonine, tryptophan, and valine must be in the diet and must be present in near-balanced amounts to furnish an acceptable amino acid pattern before fish flesh can be formed. These indispensable amino acids are then determinates for success or failure of any fish husbandry program. Qualitative amino acid requirements have been determined using amino acid test diets with whole-egg protein amino acid pattern devoid of the test amino acid. Low growth resulted when any of the 10 indispensable amino acids were absent from the diet (Halver et al. 1957). In contrast, normal growth occurred when any of the other dispensable amino acids were absent from the diet. Another technique confirmed that the indispensable amino acids could not be synthesized from [$^{14}$C]-uniformly labeled glucose (Cowey and Walton 1989). Fish were given interperitoneal injections and radioactivity was measured from tissue protein isolated and hydrolyzed to release the constituent amino acids. Those amino acids containing the radioactive material were obviously synthesized from other metabolites by the fish and were not essential dietary constituents. Conversely, those amino acids that did not contain radioactivity could not have been synthesized by the fish. These were classified as indispensable and confirmed exactly the growth experiment studies with test diets.

Quantitative amino acid requirements have been determined using protein reference diets or by analysis of dietary ingredients in fish feed ingredient mixtures. Some experiments have shown that the protein requirement increased in salmonids as the salinity increased, and that the rainbow trout requirement of 40% of the diet as protein at 10 parts per thousand (‰) salinity increased to 45% of protein in waters of 20‰ salinity. This same phenomenom was observed in coho salmon on feeding trials that measured brackish water saline content (Zeitoun et al. 1974).

Test diets have been prepared that enable increments of one amino acid at a time to be fed at different diet treatments in feeds containing some whole protein components to stimulate the digestive and absorptive processes (DeLong et al. 1962). Broken line plots of growth response versus different diet treatments produced inflection points for each of the 10 indispensable amino acids when the test diets contained arginine (2.5%), histidine (0.7%), isoleucine (1.0%), leucine (1.5%), lysine (2.1%), methionine (0.5%), phenylalanine (2.0%), threonine (0.8%), tryptophan (0.2%), and valine (1.5%). Subsequent experiments have shown a sparing action of cystine on the methionine requirement, and of tyrosine on the phenylalanine requirement. The combined methionine and cystine requirement appears to be about 1.5% of the protein, with the best growth observed when 1% of cystine and 0.5% of methionine in the protein component was fed to salmonids (Halver et al. 1959). About one-third of the phenylalanine growth requirement could be spared by L-tyrosine in some experiments. The toxic effect of gross imbalance of some of the indispensable amino acids was also observed. An inhibition of growth and diet performance occurs when either isoleucine was three times in excess of leucine, or when three times as much leucine was fed with isoleucine held at the requirement level. High intake of valine also inhibited growth in salmon (Wilson 1989).

Mixtures of amino acids have been tested and little growth was obtained in salmonids when three or more DL forms of indispensable amino acids were incorporated in the diet. D-Amino acid oxidase has been isolated and measured in young salmon and trout, and in actively feeding salmon in the marine environment. D-Amino acid oxidase activity varies between fish and between the seasons of the year in which samples were obtained and processed. Therefore, toxic effects from these enantiomorphic forms of amino acids can be expected (Mertz 1972; Wilson 1989).

The nitrogen sources for fish have been examined and little sparing effect on the whole-egg protein requirement was obtained with urea or diammonium citrate used as nitrogen sources. Arginine could be used effectively to spare dispensable amino acids protein, but glycine had only a minor sparing effect on protein requirements in salmon (DeLong et al. 1959). Thus, fish react differently from other experimental animals in their ability to use simple nitrogen sources as intermediates for dispensable amino acid synthesis. Arai (1981) used AE ratios (essential amino acid content divided by total essential amino acid content including cystine and tyrosine) of whole body coho salmon fry to formulate test diets for this fish. Many reports show that amino acid test diet growth responses are lower than comparable diets containing intact protein, even when a large portion of the protein component in the diet is made up of native protein. Fish fed the casein diet supplemented with amino acids to simulate the AE ratios of whole body tissue showed much improved growth and feed efficiency. These AE ratio diets were used to design test diets for cherry or masu salmon (*Oncorhynchus masou*) and amago salmon (*O. rhodurus*) fry. In both species the diets containing casein, plus amino acids to simulate the AE ratio of cherry salmon eggs or white fish meal, produced a better growth response (Wilson 1989).

*Arginine.* Salmon appear to have the highest requirement for arginine at about 6% of the dietary protein, whereas other species have reported values between 3% and 4%. This may be due to a limitation in the urea cycle enzyme synthesis of arginine from other nitrogenous sources in chinook salmon or coho salmon. The enzymes are present for the arginine-ornathine-citruline cycle but the activities appear to be higher in rainbow trout. Thus, this difference may explain the reports of about 4% of protein for rainbow trout and about 6% of protein for chinook or coho salmon as the arginine requirement. The arginine requirement may be influenced by salinity; 3.3% of protein as arginine in rainbow trout in fresh water decreased to 2.8% of protein in 20‰ salinity, and to 2.2% of protein as arginine in full strength seawater (Kaushik 1979). These data then indicate that the protein requirement should decrease as salinity increases. In contrast however, rainbow trout fingerlings were reported to require 40% of protein for optimum growth at 10‰ salinity and 45% whole egg protein at 20‰ salinity (Zeitoun et al. 1973). The arginine requirements are summarized in Table 5.

*Histidine.* Muscle carnosine concentrations have been shown to be altered by dietary histidine in both the chick and in the chinook salmon. In a histidine-deficient diet, muscle carnosine was depleted (Lukton 1958). Histidine requirements of salmonids are shown in Table 5.

*Isoleucine.* The requirement for isoleucine appears to be between 2 and 2.6% of the protein for most species studied. High levels of isoleucine depressed growth and when the isoleucine to leucine ratios exceeded two to one, growth inhibition occurred (Wilson 1989). Isoleucine requirements of salmonids are listed in Table 5.

*Leucine.* The leucine requirement appears to be about 3.3% to 4% of the protein. Leucine may facilitate the tissue uptake of branch-chain amino acids and it is important to have at least equal amounts of leucine to isoleucine in the diet ingredients. Much higher levels of leucine on the order of three to five times the isoleucine content were required before growth inhibition could be measured. Leucine requirements of salmonids are listed in Table 5.

*Valine.* This branch-chain amino acid appears to be required between 3 and 4% of the protein. High levels of valine in the diet also inhibit growth, with very little growth observed when 10 to 15% of the protein was valine. Valine requirements of salmonids are listed in Table 5.

*Lysine.* Most salmonids require about 5% of their protein as lysine, although some reports indicate rainbow trout may have a lower lysine requirement. The dietary

TABLE 5

Estimated amino acid requirements of salmonids as a percentage of the diet[a].

| Amino acid | Species | Requirement (% of diet) | % of dietary protein | Reference |
|---|---|---|---|---|
| Arginine | Chinook salmon | 5.8-6.0 | 2.4/40 | Klein and Halver 1970 |
| | Coho salmon | 5.8/6.0 | 2.3/40 | Klein and Halver 1970 |
| | Chum salmon | 6 | 2.6/40 | Akiyama 1987 |
| | Rainbow trout | 3.3-5.9 | 1.4/35 | NRC/NAS 1993 |
| Histidine | Chinook salmon | 1.8 | 0.7/40 | Klein and Halver 1970 |
| | Coho salmon | 1.8 | 0.7/40 | Klein and Halver 1970 |
| | Chum salmon | 1.6 | 0.7/40 | Akiyama 1987 |
| Isoleucine | Chinook salmon | 2.2 | 0.9/41 | Chance et al. 1964 |
| | Chum salmon | 2.4 | 1.0/40 | Akiyama 1987 |
| | Lake trout | 2.2 | 0.6/27 | Hughes et al. 1983 |
| Lysine | Chinook salmon | 3.9 | 1.6/41 | Chance et al. 1964 |
| | Chum salmon | 3.8 | 1.5/40 | Akiyama et al. 1985 |
| | Rainbow trout | 4.2 | 1.9/45 | Walton et al. 1984 |
| Methionine w/cytsine | Chinook salmon | 4.0 | 1.6/40 | Halver et al. 1959 |
| | Chum salmon | 3.0 | 1.2/40 | Akiyama 1987 |
| | Rainbow trout | 2.9 | 1.0/35 | Kim et al. 1984 |
| Phenylalanine w/tyrosine | Chinook salmon | 5.1 | 2.1/41 | Chance et al. 1964 |
| | Chum salmon | 6.3 | 2.5/40 | Akiyama 1987 |
| Threonine | Chinook salmon | 2.2 | 0.9/40 | DeLong et al. 1962 |
| | Chum salmon | 3.0 | 1.2/40 | Akiyama et al. 1985 |
| Tryptophan | Chinook salmon | 0.5 | 0.2/40 | Halver 1965 |
| | Coho salmon | 0.5 | 0.2/40 | Halver 1965 |
| | Chum salmon | 0.7 | 0.3/40 | Akiyama et al. 1986 |
| | Rainbow trout | 0.6 | 0.2/35 | Wilson 1989 |
| Valine | Chinook salmon | 3.2 | 1.3/40 | Chance et al. 1964 |
| | Chum salmon | 3.0 | 1.2/40 | Akiyama 1987 |

[a] adapted from Wilson (1989) and NRC/NAS (1993)

arginine-lysine interaction common in other animals has not been demonstrated in fish when either excess lysine was fed in diets adequate or marginal in arginine, or when excess arginine was fed in diets adequate or marginal in lysine. One report suggested that increasing dietary lysine intake may affect plasma arginine and urea levels in ammonia excretion, and may have been due to a decrease in arginine degradation as the level of dietary lysine was increased (Kaushik and Fauconneau 1984). Lysine requirements of salmonids are listed in Table 5.

*Phenylalanine and Tyrosine.* Phenylalanine and tyrosine, the aromatic amino acids, are both needed for proper protein synthesis. Fish can convert phenylalanine to tyrosine or utilize some dietary tyrosine to meet metabolic demands for this amino acid. The requirement for phenylalanine listed in Table 5 has been determined with either some tyrosine present in the diet or diets devoid of tyrosine. This requirement appears to be about 5% of the protein when 0.4% tyrosine is present in the diet and may be greaterin diets devoid of tyrosine. Fish need both phenylalanine and tyrosine for metabolic activity, and as only part of the phenylalanine can be converted to tyrosine and still meet the phenylalanine requirements of the animal, it is important to have some tyrosine present in the finished feed (Wilson 1989).

*Methionine.* Methionine or total sulphur amino acid requirements are listed in Table 5. Some species differences may occur with chinook salmon requiring the highest level at about 4% of the protein as methionine, whereas the rainbow trout may require only about 3% of the protein as methionine. In purified diets deficient in methionine, bilateral cataracts have also been observed in rainbow trout. The cataracts were prevented by supplementing the isolated soybean protein diet with methionine. This deficiency sign appears to be unique to this species. Dietary cystine reduces the amount of dietary methionine necessary for maximum growth. The cystine replacement value for methionine on a sulphur basis appears to be about 40% for rainbow trout. The methionine hydroxy analogue is only partially effective as a methionine source, and taurine was poorly utilized as a sulphur source for rainbow trout. D-Methionine has been shown to be as active as L-methionine component for rainbow trout diets (Wilson 1989).

*Threonine.* The requirement for threonine in young chinook salmon was the same when determined at temperatures of either 8 or 15°C. The requirement appears to be between 2.2 and 3% of the protein for most salmonids. Threonine requirements are presented in Table 5.

*Tryptophan.* The tryptophan requirement of about 0.5% of the protein appears to be adequate for most fishes studied. The chum salmon (*Oncorhynchus keta*) requirement may be reduced upon more careful examination of the data presented to date. Tryptophan deficiency results in temporary scoliosis and lordosis in sockeye salmon, but not in chinook salmon fed tryptophan-deficient diets. This same lordosis and scoliosis has been observed in tryptophan-deficient rainbow trout. These deformities were reversed rapidly when the fish were fed adequate dietary tryptophan and appeared to be related to depletion of 5-hydroxy-tryptophan in the body or brain. Other tryptophan deficiencies in rainbow trout include renal calcinosis, fin erosion, cataracts, and short gill opercles. Increased levels of calcium, magnesium, sodium, and potassium were also found in liver and kidney (Wilson 1989). Tryptophan requirements are listed in Table 5.

### *Biological Availability*

Protein digestibility and amino acid digestibility may be measured using inert markers and collection of fecal wastes. Digestibility values for different protein components and different feed ingredients vary widely. Highly purified proteins such as casein, gelatin, egg albumin, or lyophilized cod muscle, cannot be compared with proteins found in most unprocessed feedstuff components. Feedstuff processing, heat treatment, and partial denaturation of proteins improve diet protein digestibility. Fortunately, digestibility measurements on fish meal, meat and bone meal, feather meal, and other rich protein sources indicate ready digestion by the carnivorous salmonid. Other ingredients such as oil seed-base proteins, high temperature-dried fish meals, and cereal grain proteins indicate these materials must be carefully recalculated for digestible protein content in the formulation of the finished ration. The amino acids in these proteins must not only be present, they must be digestible, hydrolyzed into a form that can be absorbed, and then present in adequate balanced amounts to allow tissue protein synthesis. Analysis of fecal protein waste from these diet ingredients will indicate those amino acids that are lost from the chemical composition as the protein actually digested to provide the amino acids required for growth (*see* page 616).

A practical method to estimate indispensable amino acid requirements of fish is to examine the proportion of each amino acid present in the protein component of the ration. An example is the amino acid requirement of salmonids when 40% of the diet as protein is fed to young rapidly growing fish at 10°C. A remarkable and consistent percentage of each is obtained when the gram percent quantities of the individual amino acids are compared between rainbow trout, chinook salmon, and coho salmon. Slight differences are observed only between histidine and isoleucine requirements and phenylalanine, threonine, and valine requirements. Only lysine shows a major difference among species when the gram percent quantities are extended into percent of dietary protein. The gram percent of the indispensable amino acid requirement may drop as the fish grow larger, but so does the protein requirement. The muscle mass of fish reflects the amino acid needs for synthesis, thus the percent of amino acid in the dietary protein is a reasonable measurement of amino acid requirement (Wilson and Halver 1986).

Most of the amino acid quantitative studies have been conducted with very digestible protein components to furnish the amino acids, or with amino acid mixtures that will guarantee rapid absorption of the test nutrient fed. This technique is logical to catalogue the indispensable amino acid requirements and to measure the sparing effect of certain dispensable amino acids on these requirements for various species of fish to be reared in defined experimental conditions. More work needs to be done with important commercial types of fish confined in fish husbandry reproduction facilities to provide information on the indispensable amino acid requirements and a good pattern for fish rations. This will allow least-cost computer formulation of practical diets and improve the efficiency and economics of the industry. However, when testing amino acid patterns in practical rations, one must first calculate the portion of the protein that is digested and utilized (Wilson 1989).

## LIPIDS

Salmonids have very active lipases; therefore dietary lipids can serve as energy sources and also as sources of essential fatty acids. Essential fatty acids have cellular functions and must be present in the diet to prevent deficiency signs. Salmonids have a specific requirement for n-3 fatty acids, which are present in phospholipid components of cellular membranes (Dey et al. 1993). The degree of unsaturation of the essential fatty acids determines the melting point and subsequently the flexibilities of tissue membranes when exposed to the low water temperatures of the salmonid environment. Rainbow trout were shown to have a specific requirement for n-3 fatty acids. Linolenic acid (C18:3 n-3) could be used but eicosapentaenoic acid (C20:5 n-3) and similar polyunsaturated fatty acids were more effective as essential fatty acid sources. Trout and salmon have the ability to elongate fatty acids but are deficient in their ability to unsaturate some fatty acids (Lee and Sinnhuber 1972).

Essential fatty acid requirements for selected salmonids are shown in Table 6. Rainbow trout require about 1% of 18:3 n-3 fatty acid in the diet and some reports indicate higher levels of n-3 type fatty acids are required at higher dietary lipid levels. Coho salmon have been reported to require between 1 and 2% of n-3 fatty acids to prevent deficiency signs at normal growth rates. Chum salmon fry required about the same levels of n-3 fatty acids in the diet when linolenic acid was used, but the requirement was only about 0.5% when longer chain n-3 fatty acids were used. The essential fatty acid deficiency signs are elevated muscle water content, caudal fin erosion, shock syndrome, swollen and pale livers, and high mortality. Linoleic acid (n-6) may also be required, but the research results to date have not confirmed the requirement for this type of fatty acid in coldwater fish, whereas it has been shown to be essential in certain warmwater fishes. The requirements for essential fatty acids in the marine environment may be higher. Certain marine fish have been reported to require about 3% of the diet as polyunsaturated n-3 type fatty acids (Sargent et al. 1989; NRC/NAS 1993).

It is well documented that the fatty acid profile in the stored neutral lipids can be altered by the fatty acid profile of ingested lipids. This enables salmon and trout to have their lipid stores change with respect to dietary fatty acid intake. The phospholipid

**TABLE 6**

Estimated essential fatty acid requirements of selected salmonids[a].

| Species | n-6 (% diet) | n-3 (% diet)[b] | Reference |
|---|---|---|---|
| Chum salmon | 1% linoleic | 1% linolenic | Takeuchi & Watanabe 1982 |
| Coho salmon | | 1-2.5% linolenic | Yu & Sinnhuber 1979 |
| Rainbow trout | | 1% linolenic | Castell et al. 1972 |
| | | 0.8% linolenic | Watanabe et al. 1974 |

[a] adapted from Lee and Sinnhuber (1972) and NRC/NAS (1993)

[b] 20:5 n-3 (eicosapentaenoic acid) may be more effective than 18:3 n-3 (linolenic acid)

components remain relatively fixed by the genetic information incorporated into phospholipid synthesis and subsequent membrane synthesis, but the neutral lipid stores, which are used primarily as energy reserves for the animal, can be changed. It is technically possible to produce a fish with a ratio of n-3 to n-6 polyunsaturated fatty acid that is optimal for human nutrition. The pathways for elongation and unsaturation of fatty acids are outlined in Figure 6. A more thorough discussion of the roles of fatty acids in fish nutrition is presented by Sargent et al. (1989).

## MINERALS

Calcium, phosphorus, magnesium, and iron are required in milligram quantities in the diet for salmonids, while other minerals are required in microgram quantities. As calcium is readily sequestered from the water, major emphasis should be focused upon an adequate phosphorus intake.

### *Phosphorus*

Bioavailability of phosphorus compounds varies considerably. Many of the agricultural source feedstuffs contain phosphorus firmly bound to phytic acid in the phytin moitity, which is unavailable to fish because salmonids lack an effective phytase enzyme to hydrolyze this compound. Fish and animal meal sources, however, contain readily available phosphorus and generally satisfy the phosphorus requirements in diet

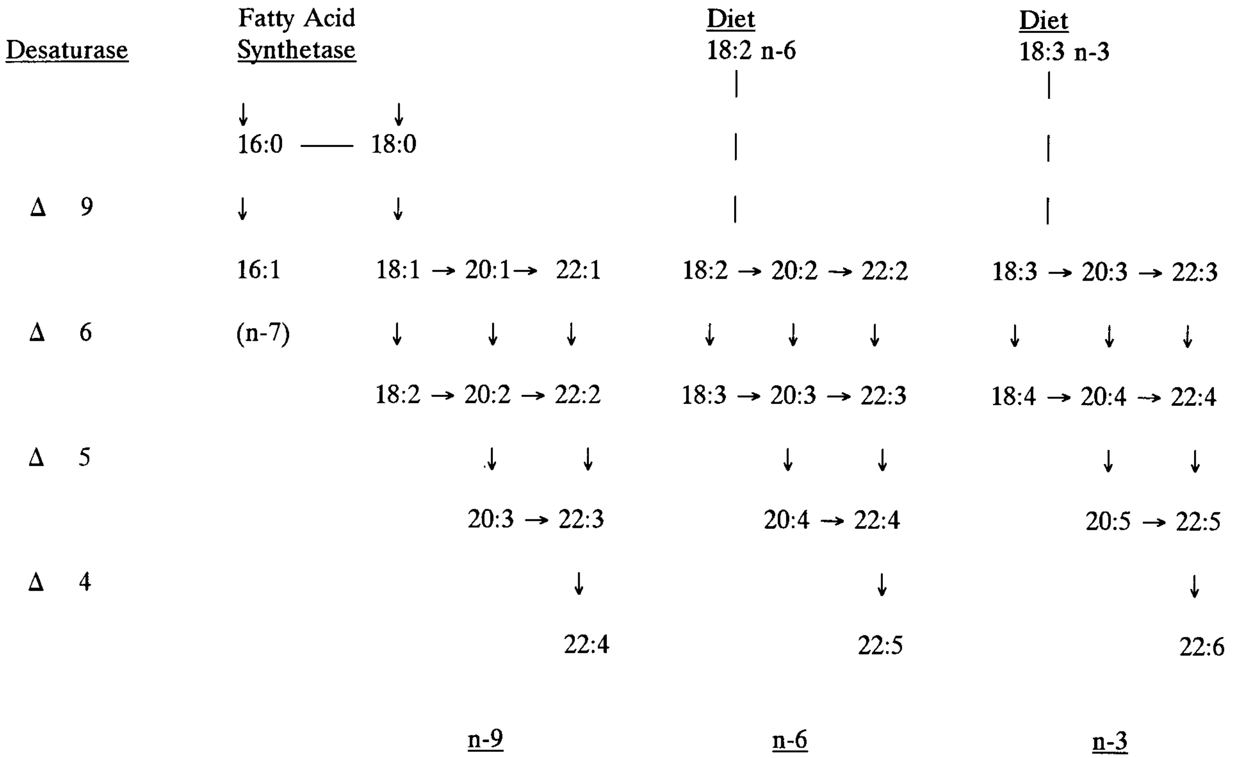

FIGURE 6. Pathways of fatty acid elongation and unsaturation (adapted from Sinnhuber *in* Neuhaus and Halver 1969).

formulation. Deficiency signs are anorexia, poor feed conversion, low bone mineralization, skeleton deformity, spongy vertebrae, and other skeletal abnormalities. The phosphorus requirements for trout and salmon are listed in Table 7 (Lall 1989).

### *Magnesium*

Magnesium is generally not a major problem in fish diet formulations because it is a major component in most skeletal tissue and in the marine environment, it is present in the salts. Magnesium is essential for many fundamental enzymatic reactions in intermediary metabolism. These include phosphate transfer, acetyl coenzyme A, and fatty acid oxidation in various amino acid synthetases. It is also involved in neuromuscular signal transmission and is important in fish respiration. In fresh water, fish can derive magnesium either from the environment or from dietary sources. In seawater, magnesium is obtained by drinking and excess magnesium is excreted through the kidneys. Magnesium deficiency is listed in Table 8 and is a result of impaired enzymatic function in both anabolism and catabolism (Lall 1989).

### *Iron*

Iron is involved in cellular respiration and acts in oxidation-reduction activity in electron transport. Iron is generally absorbed from the digestive tract in the reduced ferrous state, and the vitamin C content of the diet enhances iron absorption by maintaining it in the reduced state. Iron is transported in the blood by transferrin, and is stored in erythrocytes as heme compounds and in the liver, spleen, and head kidney. Iron deficiency signs are listed in Table 8, and deficiency is generally manifested by hypochromic microcitic anemia (Lall 1989).

### *Zinc*

Zinc is an essential element for salmonids and plays an important role in several metalloenzymes. It is also a catalyst for regulating activity of zinc-dependent enzymes. Many zinc metalloenzymes have been identified including carbonic anhydrase, alkaline phosphatase, and alcohol dehydrogenase. Zinc is involved in metabolism of carbohydrate, lipid, and protein. It is also involved in nuclear proteins and in the metabolism of prostaglandin. Fish can absorb zinc from both water and dietary sources and high concentrations of zinc inhibit respiration. Salmonid fry in high zinc content water have yolk coagulation and impaired fry development. Zinc deficiency is listed in Table 8, compared with deficiency identified in other fishes (Lall 1989; NRC/NAS 1993).

TABLE 7

Estimated mineral requirements of salmonids as mg/kg of diet[a,b].

| Mineral | Requirement<br>Pacific salmon | <br>Rainbow trout |
|---|---|---|
| Magnesium | 500 | 500 |
| Phosphorus | 6000 | 6000 |
| Potassium | 900 | 700 |
| Sodium | | 6000 |
| Copper | 5 | 3 |
| Iodine | 1.1 | 1.1 |
| Iron | 45 | 60 |
| Manganese | 15 | 13 |
| Zinc | 40 | 30 |
| Selenium | 1.0 | 0.5 |

[a] fish reared in fresh water

[b] adapted from Lall (1989) and NRC/NAS (1993)

### *Manganese*

Manganese functions as a cofactor activating a large number of enzymes that form metal enzyme complexes. Kinases, transferases, hydrolases, and decarboxylases are involved. Glycocyl transferase is one example of a specific manganese-activated enzyme. Arginase, pyruvate carboxylase and superoxide dismutase contain manganese. Manganese can be obtained from the water or from the gastrointesinal tract in fish, but manganese is more efficiently absorbed from the feed ingredients and its absorption may be depressed by high levels of dietary calcium or phosphorus. Deficiency signs listed in Table 8 generally cause reduced growth and skeletal abnormalities.

### *Copper*

Copper is an essential element for all animals studied to date. Copper is involved with cytochrome oxidase in the electron transport chain as well as other copper-containing enzymes such as superoxide dismutase, lysoloxidase, dopamine betahydroxylase, and tyrosinase. It is necessary for melanin production and cross-linking of elastin and collagen components. In fish, blood copper is present as the copper-protein complex, ceruplasmin. Deficiency signs are listed in Table 8.

TABLE 8

Mineral deficiency signs in salmonids (species in parentheses)[a].

| Mineral | Deficiency signs |
|---|---|
| Calcium | Reduced growth and poor feed conversion (1) |
| Phosphorus | Reduced growth (1,3), poor feed conversion (1,3,5), poor bone mineralization (1,3) |
| Magnesium | Reduced growth (1), anorexia (1), sluggishness (1), nephrocalcinosis (1), cataracts (1), degeneration of muscle fibers and epithelial cells of pyloric caeca and gill filaments (1), skeletal deformity (1), reduced bone ash and bone magnesium concentration (1), high mortality (1) |
| Iron | Hypochromic microcytic anemia (2), low hematocrit and haemoglobin levels (1,3) |
| Zinc | Reduced growth (1), short-body dwarfism (1), cataract (1), high mortality (3), reduced bone manganese concentration (2,3), poor hatchability of eggs (1,2,3), abnormal tail growth (1) |
| Copper | Reduced liver copper-zinc-superoxide dismutase (3), and heart cytochrome C oxidase activity (3) |
| Selenium | Muscular dystrophy (3), exudative diathesis (1), reduced glutathione peroxidase activity (1,3) |
| Iodine | Thyroid hyperplasia (1,2,5) |

| | |
|---|---|
| (1) | Rainbow trout |
| (2) | Brook trout |
| (3) | Atlantic salmon |
| (4) | Chum salmon |
| (5) | Chinook salmon |

[a] adapted from Lall (1989)

### *Selenium*

Selenium is an integral portion of glutathione peroxidase, the enzyme halting lipid peroxide formation and protecting polyunsaturated fatty acids from oxidation. High levels of selenium in the diet cause toxic reactions, but low levels are essential for the protection of cells and membranes from oxidative damage. The deficiency signs are listed in Table 8, with exudative diathesis and muscle dystrophy occurring in trout and salmon. The dietary requirement for salmonids may be much higher than allowed by the U.S. Food and Drug Administration at the current regulation of 0.1 part per million. Toxicity occurs when dietary intake exceeds 10 to 15 mg/kg feed. But when 1 to 2 mg selenium/kg feed is fed to anadromous fish such as coho salmon, Atlantic salmon, and rainbow trout, better survival occurs during smolting and subsequent conversion to the saltwater environment (Lall 1989; NRC/NAS 1993).

### *Iodine*

Iodine is essential for the biosynthesis of thyroid hormones. Fish can obtain iodine from the water but adequate iodine should be present in the diet to assure iodide binding to plasma proteins. The deficiency signs listed in Table 8 generally manifest as hypothyroidism or goiter. This deficiency was first described more than 80 years ago by Gaylord and Marsh (1914). Increased iodine requirements may occur during smoltification, and certain goitrogenic substances may influence the iodine requirement and stimulate hyperthyroidism. In the freshwater environment adequate iodine must be present in the diet, but when fish meals are a major component of the diets, adequate iodine probably is present. In the marine environment, algae and zooplankton contain large amounts of this essential element, and marine fish tissue has heavier concentrations of iodine than fish from freshwater environments.

Other trace elements include cobalt, which is present in the structure of vitamin $B_{12}$, chromium, which is part of several biologically active compounds, boron, molybdenum, fluoride and all the ultra-trace elements. All may be required but specific deficiency signs and requirements have not been elucidated (Lall 1989).

The bioavailability of trace elements are affected by the concentration of other polyvalent ions in the diet. One typical example is high calcium diets that reduce the bioavailability of zinc, with subsequent development of cataracts in fish fed white fish meals with high salt content. The functions and bioavailability of selenium are affected by ascorbic acid content of the diet, and vitamin C present in the tissue for this biologically active reducing compound protects the oxidation state of selenium in glutathione peroxidase. Similar effects can be found in copper functions in the cytochrome C electron transport system, in absorption of iron in the reduced state from the gut, and probably in the chromium and other polyvalent ions present in the tissues. Finally, sodium and potassium levels in the diets and the chloride content, which is involved in osmoregulation within and between tissues in fish in fresh water, play a major role in fish physiology. The sodium and potassium content is generally adequate in most diet mixtures except when highly purified test diets are used. Anadromous salmonids of course migrate from a hypotonic to a hypertonic environment with major physiological changes that affect inter- and intra-cellular concentrations of both sodium and potassium. Dietary supplementation with sodium and potassium salts may facilitate smoltification but normally adequate amounts of these elements are present in diet mixtures. More thorough reviews of mineral requirements and metabolism are found in Lall (1989) and NRC/NAS (1993).

## ANTI-NUTRITIONAL FACTORS IN FEEDSTUFFS

Most feedstuff materials are processed for the poultry industry and the heat used to denature soybean trypsin inhibitor may be acceptable for poultry diets, but are unacceptable for fish diets when traces of the inhibitor remain. Fullfat soybean has recently been developed as an excellent protein and lipid source, but heat treatment must be carefully controlled and tested to be assured the soybean trypsin inhibitor is completely inactivated. In addition, some of the carbohydrate materials in soybeans are not readily accepted by chinook salmon but may be used in diets for trout and other salmon. Insufficient heating will interfere with protein digestion, but overheating also leads to low digestibility and amino acid availability.

Hemaglutens are also present in many agricultural products and can bind to free sugars or to polysaccharide residues and to cell membranes, causing agglutination properties. Legumes contain high levels of these hemaglutens and heat treatment will inactivate these proteins. Some inactivation occurs also in the acid stomach of salmonids by pepsin.

Phytic acid, the hexaphosphate of inositol, is present in many cereal products and oilseed meals. These phytates have the capacity to bind divalent cations, such as calcium, magnesium, and zinc, and most of the phosphorus plus some of the cross-linked calcium and magnesium salts are not available to the animal. Phytoestrogens occur in many agriculture compounds, including cereal grains, soybeans, and rapeseed. These compounds may have an impact upon estrogen-androgen balance and, thus, on fish reproduction.

Glucoinsinolates, or thioglucoside, occur in the cabbage and broccoli family, as well as in rapeseed, mustard seed, and greens. These are goitrogens which inhibit thyroid activity and affect organic binding of iodine. Rapeseed meal, a low cost protein source for salmonid diets, contains thiocyanate, which also interferes with iodine function. Rapeseed meals also contain other toxins, as well as high fiber content. The erusic acid and tannins interfere with some fish metabolic processes causing cardiac lesions. More recently developed low erusic acid rapeseed varieties have been used successfully in salmonid diets, but carcass analysis showed an increase in 22:1 n-9 fatty acids at the expense of 22:6 n-3 fatty acids (Hendricks and Bailey 1989).

Many alkaloids occur in angiosperm plants. Rainbow trout exposed to these alkaloids develop liver necrosis and severe liver toxicity. Gossypol is present in cottonseed, and cottonseed meal has an excellent amino acid profile and has been used as a component in fish diets. High levels in the diet result in liver necrosis and ceroid deposition in the liver, spleen, and kidney. In addition, low levels of gossypol in the diet increased the carcinogenicity of the aflatoxins found in moldy cereal grains and oilseed meal products.

Cyclopropene fatty acids, such as sterculic and malvalic acids are also present in some cottonseed meal. Levels in excess of 20-30 mg/kg dry diet result in hepatocyte necrosis and appearance of fiber-like material in the hepatocyte cytoplasm. The cyclopropene fatty acids also enhance the effects of the mold toxins; for example, sockeye salmon developed hepatoma only when both aflatoxin $B_1$ and cyclopropene fatty acids were present in the diet, whereas the same levels of aflatoxin $B_1$ alone did not induce hepatoma. Young fish seem more susceptible than older fish to cyclopropene fatty acids, which may be due to the lower levels of developed MFOs in young fish.

Many other toxins occur in plant materials, such as favatoxin, cyanogenic glycosides, oxalates, D-amino acids, vomitoxins, and indols. These all must be detoxified by the fish's metabolic system, and therefore invoke increased activities of MFOs and other detoxifying systems. The concomitant energy demands of detoxification result in less efficient dietary use when diets containing these compounds are fed (Roberts and Bullock 1989; Hendricks and Bailey 1989).

Many mycotoxins occur in improperly stored diet material. The role of aflatoxins in rainbow trout hepatoma is well documented with only trace amounts of this material resulting in significant numbers of hepatomas in fish within one year. Aflatoxicosis in rainbow trout appeared at $LD_{50}$ levels of less than 1 mg/kg for aflatoxin $B_1$, and about 2 mg/kg for aflatoxin $G_1$. In contrast, the $LD_{50}$ for aflatoxin $B_1$ was between 5-10 mg/kg in coho salmon (Halver and Mitchell 1967; Halver 1969). Hence the toxicity of these mycotoxins varies between species with rainbow trout as the most sensitive animal tested to date. Much lower levels in oral doses of about 0.5-1.0 part per billion in the diet will produce a significant incidence of hepatoma in rainbow trout (Halver 1969). When these low levels of the aflatoxins are present in diets containing cyclopropenoid fatty acids, even higher levels of liver hepatoma appear (Hendricks and Bailey 1989). Thus it is important to minimize mycotoxin and other toxicant content in salmonid fry diets. Larger fish with better developed MFO systems appear to be less sensitive to these compounds. In addition, aflatoxin, ochratoxin, rubratoxin, T-2-toxin and vomitoxin have all been identified in grain products and dry malt feed. Most of these have acute toxcitity at high levels in the diet, and low levels interfere with diet efficiency and growth.

In addition to the plant and animal toxins, many organic materials, pesticides, polychlorinated biphenyls, nitrosamines, heavy metals, hormones, and preservatives may be present in the feed. Each of these should be examined and low levels of these toxicant materials eliminated in the finished feed. More thorough discussions of the various adventitious toxins which may occur in fish feeds are found in Friedman and Shibko (1972) and Hendricks and Bailey (1989).

## FEEDS FOR SALMONIDS

At least eight different types of manufactured fish feeds are used in salmonid diets. The ultimate application of the nutrient requirement studies and the nutrient profiles present in various feedstuffs is the formulation of an economical efficient diet to rear the individual species in the specific environment of the husbandry. The feedstuffs nutrient data bank today contains nearly 10,000 line items of agricultural products available for fish feeds. This same data base contains 60 columns of nutrients that have been catalogued as required for different species of salmonids. Unfortunately, most of these materials are not correlated with digestibility coefficients for protein, carbohydrate, lipid, and mineral content. As these voids become completed for the different species of salmonids, for different sizes of fish, and for different husbandry and environmental conditions used in fish husbandry, it will become increasingly more useful to use least-cost computerized formulations to satisfy the nutrient requirements of these fishes. More than 100 line items of commonly used ingredients are currently available that have digestibility coefficients for dry matter, energy, proteins and amino acids, and when used they do result in reasonably acceptable economic diets provided that adventitious toxins have been eliminated from the formulations (Halver 1989*a*).

Fry diets can be made from meat-meal mixtures, denatured or bound together with binder, and supplemented with vitamins and lipids as needed to satisfy the specific requirements. Most salmonid fry diets are presently manufactured as microparticulate diets or as mashes made from mixtures of processed fish meal plus plant protein meals, marine oils, trace minerals, lipids, and vitamins. As fry grow, larger crumbles made from compressed pellets that have been pulverized and screened to size are used. Flake feeds are sometimes used for very small fry, with subsequent transition to larger particle size materials as the fry develop. As the size of the fish increases, particle size increases until 1- or 2-mm pellets can be fed. At this stage, either dry compressed pellets made from computerized formulations of feedstuffs available, or expanded pellets made by steam-treating mash mixtures of feedstuffs ingredients and subsequently drying to the desired moisture content, will allow either floating or slowly sinking pellets to be made. As the fingerlings grow, larger pellets are used with decreasing protein content and more carbohydrate and lipid components. Growout pellets for final stages of production contain less protein and a wider variety of feedstuff ingredients, and are often coated with additional lipid to increase the energy content of the diet and maximize growth response.

The eight common types of diets manufactured for salmonids are summarized as follows:

(1) Moist or semi-moist mixtures, containing meats or pasturized fish products and bound together or denatured with assorted material fed to fry or extruded in large amounts to feed to larger fish.

(2) Microparticulate diets made by internally binding mixtures of feedstuffs then pulverizing and screening to size for fry feeds. Trace minerals, vitamins, and lipid components are added to balance the nutrients required by the fry.

(3) Microencapsulated diets made by forming an external coating with protein, lipid, or fibrous material, then feeding these small capsules to fry. Amino acid balance, lipid additions, vitamin supplements and trace mineral supplements are used in the microencapsulation process.

(4) Flaked feeds manufactured by flash-drying slurries of meat-meal mixtures supplemented with lipids, vitamins, and trace minerals that are dried, pulverized, and screened to proper size for fry.

(5) Crumbles manufactured from compressed pellets made from mixtures of feedstuffs supplemented with vitamins, minerals, and lipids. The pellets are crumbled and screened to size for small fish, and larger crumbles are used as fish increase in size.

(6) Small (1 to 2 mm) pellets made by compressing feedstuffs mixtures supplemented with vitamins, trace minerals, and lipids, and then extruded through a die under high pressure. These are fed to larger fry with pellet size increasing as fish grow in size.

(7) Expanded pellets ($\geq$2 mm) manufactured from feedstuffs mixtures supplemented with vitamins, trace minerals, and lipids, and then extruded under high steam pressure through special dies. After expansion these are cooled and screened to size as either floating or slowly sinking pellets for fingerling production. These pellets, because of superior binding characteristics and water stability, are becoming the most commonly used in fish husbandry today.

(8) Rolled pellets made by rolling ground meal mixtures of ingredients under a water spray, collecting the particles, drying these and screening them to size for floating or slowly sinking pellets. The sizes can be collected from fry feeds through growout pellets.

Growout feeds consist of larger pellets 4-6 mm in size, and can be manufactured as cold compressed pellets, steam-treated compressed pellets, or as expanded feeds. The expanded feeds have the additional advantage of greater water stability and are often supplemented with top dressings of lipid to increase the caloric density to insure rapid fish production. Top dressing of cold or steam-compressed pellets and of Dravo-type rolled pellets are also possible. Steam-conditioned compressed pellets are the most economical to manufacture and require less cooling and drying machinery than expanded pellets. The high manufacturing cost of expanded pellets is compensated for by greater flexibility in processing, protein and pellet density, and in improved use of the carbohydrate materials as these are partially gelatinized in the process. A number of sources provide a more complete description of feed manufacturing, feeding practices, and diet formulation (Halver 1980; Hardy 1989; Lovell 1989; Pigott and Tucker 1989). Additional general information can be found in NRC/NAS (1993).

## SUMMARY AND COMMENTS

Most of this chapter was abstracted from material contained in Fish Nutrition, 2nd Edition (Halver 1989*a*) and from the latest National Research Council (NRC) bulletin on Nutrient Requirements of Fish (NRC/NAS 1993). These recently published books contain hundreds of specific references to original salmonid nutrition experiments conducted in many laboratories during the past 50 years. The NRC bulletin on Nutrient Requirements of Fish contains more than 900 references and Fish Nutrition, 2nd Edition, contains more than 3,000 references. The reader is urged to review cited publications when specific details on experimental techniques, methods, and data are desired to add to understanding of how these nutrient requirement values were generated. As more data become confirmed for nutrient requirements of different sizes and species of salmonids reared under different environmental conditions, the International Network of Feedstuffs Information Centres (INFIC) data bank will become more useful and effective for least-cost computation of effective diets for fish husbandry.

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## Chapter 11

# BREEDING AND GENETICS

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## INTRODUCTION

### *Scope of Chapter*

In this chapter different kinds of genetic variation of salmonids will be dealt with and related to aquaculture in its widest sense; that is, any manipulation of the organisms or their environment to improve, restore or conserve the organism's value as a resource for commercial or sport fisheries or farm production. Thus intensive fish rearing as well as enhancement and sea ranching are included herein. Environmental manipulation such as fertilizing, dam building, partial feeding, or building of fish ladders, are dealt with elsewhere.

The scope of the chapter is to select valuable information from the literature with respect to genetic variation between and within salmonid species and populations and discuss their mode of inheritance, biological significance, their importance for improving performance of farmed strains under farm conditions, and the problems associated with restoration and preservation of salmonid resources. The maintenance of genetic diversity within and between populations is essential for both conservation of genetic resources and their use under controlled production in aquaculture.

Basic knowledge of genetic variation has been gathered since the germ cell lines and the laws of inheritance were discovered, although there evidently was some understanding of the significance of genetic variation long before that. Concerning salmonids, however, the real understanding of genetic variation within species is based on knowledge gathered during the last 30 years. Variation between species, however, has been known for a long time, but much confusion and incomplete understanding have been and still are widespread, and this is clearly reflected in the different scientific names used for species, subspecies and "strains" (Falconer 1960). The application of the species concept for salmonids is even more complicated by the fact that most species can easily be hybridized and even give fertile progeny (*see* below). In many cases the distinction between species and "strains" is thus obscure.

### *Historical Review of Breeding Programs in Farmed Animals*

The history of breeding is much longer than the history of genetic knowledge. Farm animals have been selected for as long as humans have been able to control the reproductive process. Artificial selection has mainly been carried out as a choice of the assumed best animals for breeding populations, and especially choice of a sire for fertilizing several dams. The genetic gain obtained in this way is dependent on both the accuracy of measurement and the degree of genetic control of the trait in question (i.e., heritability, *see* below) as well as the intensity of selection. Thus, selective breeding of farm animals was conducted long before the basic laws of inheritance were known and before the concepts of modern quantitative population genetics were defined and utilized. Modern population genetic theory has contributed enormously to the improvement of farm animal productivity (Falconer 1960; Wright 1968, 1969), and this evident success is, no doubt, the background for several studies undertaken on the quantitative genetics of fish (Gjedrem 1975, 1993; Nævdal 1981; Kinghorn 1983*a*; Refstie 1990).

However, in the past the rather vague process of domestication probably has been of greatest significance to productivity in farm animals. Domestication is also based on genetic variation, and in a farm situation the adaptation criteria are very different from those in the wild. Productivity, as the end "product", is composed of several elements including survival in a given situation as well as the ability to give the highest quantity of produce (milk, meat, or eggs, for example), which again probably will result in a higher chance of the individual being selected as a breeder for the next generation. Thus it is very difficult to distinguish between aimed selected breeding and changes caused by "domestication" in its more restricted sense.

Domestication in fish, the concept and practical importance, is discussed by Tave (1990) concluding that this process is so slow that we hardly can say that farmed fish are domesticated yet. However, changes that easily may be explained as "domestication", i.e., changes caused by adaptation to an artificial environment and not necessarily caused by planned selective breeding, are well known in intensive aquaculture. Contrary to most farmed animals the number of progeny from each pair of parents are very high in fish, and thus the possibility for changes per time unit due to such semi-natural selection is much higher. The same principles might be used in intensive fish farming breeding plans (*see* below).

### *Historical Review of Fish-breeding Programs*

Donaldson (1968) and Donaldson and Menasveta (1961) started fish-breeding programs based on mass selection of traits connected to survival and productivity. Although not very well controlled (unselected controls were lacking) selected strains showed improved growth rate, higher tolerance to low water temperatures, lower age at first maturation, higher fecundity and more accuracy in homeward migrations. Although not studied beforehand, the results imply that there must have existed considerable genetic variation that was utilized for better adaptation and higher yield in this case of "semiculture" (enhancement and sea ranching).

Moav and collaborators (Moav and Wohlfarth 1966) described experiments conducted to study the genetic basis of economically important traits in carps and construction of efficient breeding schemes for fish in general. They estimated high heritability factors for the trait body weight, but remarkably enough, no response to selection for this trait. These rather confusing results, together with relatively promising results with crossbreeding of inbred lines, caused Moav and colleagues to conclude that non-additive genetic variation was of relatively high significance in fishes, and consequently that the crossbreeding of inbred lines should be preferred in large scale breeding programs.

Carlin (1965) found that the total yield of different families used in a stocking program with Atlantic salmon (*Salmo salar*) differed considerably. Ryman (1970; 1972*a*, 1972*b*) found that these differences in yield were composed of differences in recapture frequencies, recapture weight and mortality rates, and that genetic factors caused a considerable part of these variations.

The genetic research program at the North American Salmon Research Centre (NASRC), St. Andrews, New Brunswick, Canada, is particularly noteworthy. This program originally was founded to develop improved strains for salmon ranching and for restocking depleted river populations. Although the original aim has been very difficult to accomplish due to the generally low recapture frequencies, the program has generated much knowledge about genetic variation in salmon, genetic–environmental interaction, and the use of such variation in cage culture.

Programs for genetic improvement of Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) in Norway likewise were founded on the assumption that strain and individual variations that could be used for improvement of economic traits probably exist. The programs started in the early 1970s. Genetic variation was estimated by extensive studies of strain and family performance under normal farm conditions. On this basis the genetic parameters were estimated, and selection indices and selection programs were designed.

Kincaid et al. (1987) described a selection program on rainbow trout in the U.S. starting in 1965 that had substantial success in selection for improved growth rate but not for percentage hatch and fry survival.

The scientific and practical results obtained in the programs mentioned here will be treated in some detail below (*see* page 684). All selection programs necessarily take several fish generations, and therefore the number of such long-term programs is limited despite the great interest in all forms of salmonid culture over the last 20-30 years. However, substantial knowledge on salmonids genetics has been obtained from smaller projects, likewise dealt with below.

### *Challenges and Benefits of Formal Breeding Programs for Farmed Salmonids*

Natural selection does not necessarily act in favour of productivity. In nature the criteria will be largely survival and reproduction, but under farming conditions the greater part of the selection pressure due to competition for food and reproductive success has been removed. Thus there exists a possibility of carrying out artificial selection based on criteria important for production; utilization of the genetic variation in such traits will be a challenge for the fish farming industry and its scientific supporters. The first step will be to reveal the variation and to estimate the genetic part of the underlying control mechanisms. To obtain reliable estimates, a relatively large population representing a considerable part of the species' gene pool has to be studied. To estimate the genetic component of the observed variation, performance of traits in categories of relatives (full-sibs, half-sibs or parent-offspring) must be recorded and compared. During subsequent generations selection may be achieved based on family or individual performance. To be able to distinguish between genetic improvement and improvement due to improved husbandry, unselected control populations must be maintained.

Definition of selection goals is very important, and these must be identified before the work starts. However, such goals are largely determined by market demand and may have to be modified due to changing markets. The different traits to be improved will usually be included in selection indices. Clearly the basic population should be large enough to prevent unwanted inbreeding, and the breeding population should be isolated from important diseases, especially those transferred by gametes.

Genetic selection programs will require substantial rearing facilities for many fish generations, substantial financial support, and well-planned short- and long-term experiments. On the other hand, the benefit of such program may be obtained even after one generation because the program will produce surpluses of eggs or fry of known and improved quality, and such material may be used by industry. Thus, each generation will produce material of higher value for the practical fish farmer, and data from industry may act to adjust the breeding program. Although the greatest benefit from extensive breeding programs will be obtained after several fish generations, these programs will contribute to improved production after a relatively short time, making the decision of starting such programs more attractive.

### *Outline of Other Genetic Concerns*

The salmonid breeder has to work with the genetic variability of the original wild fish that are selected as founders for the farmed strain. In principle the founders should represent as much genetic variability as possible, but because of the complicated population genetics of salmonid species, this is very difficult to achieve. Conservation of genetic resources within salmonid species and populations is an important task for preserving biological diversity. It is, however, also very important for the future of salmonid aquaculture that genetic variation in native stocks is maintained as a living gene bank where important gene complexes and traits are preserved and are available for industry when needed.

*Maintaining Integrity of Wild Gene Pools.* The homing behaviour of maturing salmonids and the often limited space and water resources for spawning indicate small gene pools with great risk of inbreeding as well as risk of genetic impoverishment and eventual extinction of local populations. Genetic impoverishment may be counteracted by gene flow between the populations as well as stabilizing selection, which tends to maintain genetic variation within the populations. The mechanisms involved in maintaining wild gene pools and population structures of the species are to a great extent unknown, although both dynamic and conservative forces are evidently involved.

Human activities may influence the wild populations of salmonids in different ways, some of which are listed below:

(1) Damage to environment due to river and lake modifications (e.g., regulated flow, erosion);
(2) Damages caused by pollution, including acid precipitation;
(3) Increased gene flow between populations due to transplanting of foreign stocks;
(4) Escapement of farmed fish into the wild environment, possibly with modified gene pools and often reduced genetic variation due to selective breeding;
(5) Selective fishing; and
(6) Overfishing.

All these influences will also alter gene pools. Strategies for successful management of both wild and artificially produced stocks of salmonids are outlined by Helle (1981), who stressed the importance of maintaining as many viable diverse wild stocks as possible, indicating that lakes and rivers with wild salmonids are the "gene banks" for salmonid fishes. Thus, transplantation of fish stocks should usually be avoided, and the requirements of the different stocks (and species) at various life history stages should be known in order to adapt hatcheries or other enhancement facilities to their needs. It should be possible to develop strategies to maintain the integrity of wild stocks and at the same time enhance the production by semi-intensive (ranching) and intensive culture.

*Restoration of Wild Stocks Reduced to Low Numbers.* When natural stocks are already depleted, restoration to former stock size will be a challenge for scientists and management agencies. Strategies will depend on the situation, but lack of information about gene pools will hamper the task. One may assume that depleted gene pools have lost their genetic variation, and have possibly suffered from inbreeding depression. Thus such populations are less fit, especially for adaptation to changing environments, and in special cases they may be very unfit. Here, transplantation of foreign gene pools could be beneficial for increasing the genetic variability of the original stock. However, little is known about the genetic background of adaptation to local environments, and the immediate effects of population mixing in nature are virtually unknown [although Altukhov (1981) presented examples of reduced total production after introduction of foreign stocks to Sakhalin rivers]. If possible, the first approximation in a strategy to restore depleted stocks should be enhancement of the natural gene pool by improving the survival of eggs and fry through artificial reproduction. If other stocks are going to be introduced, they should be chosen from watersheds with similar environmental conditions. Experience indicates that "plasticity" of adaptation and acclimation to new environments are inherent traits of salmonid stocks, and this could be due to high genetic variation in such "plastic" stocks. Another way of introducing a high degree of genetic variation in depleted or nearly extinct stocks is to transplant fish from several other stocks. Hopefully then a well-adapted "new" stock will be created in a few generations. However, the difficulties of measurement or judgement of adaptive and selective value make such strategies doubtful, and they should only be used for field experiments and in cases when the natural populations are close to extinction with little chance of unassisted recovery.

## GENETICS OF SALMONIDS

Traditionally, genetics has been defined by the degree of inheritance of specific characters from parents to offspring. The classical work of Mendel, the different studies that led to identification of deoxyribonucleic acid (DNA) as the chemical substance responsible for transference of information between generations, and recent knowledge of molecular genetics and gene technology methods, are all parts of the genetic framework which can be used to reveal the genetic structure of salmonid species and populations. The fundamental knowledge and methods are discussed below as a basis for fisheries management options and for salmonid culture.

### *Chromosome Structure of Salmonids*

Cytogenetic techniques have been used for studies of chromosomes in fish for several decades. Such investigations usually require sampling living tissues with mitotic cell activity and allowing the different chromosomes to be separated, counted and characterized for specific chromosomal structures. Samples of tissue with high division rates (gill, kidney or blood) are usually treated with a chemical which blocks mitosis. The cells are then fixed and stained to delineate chromosomes. These can then be studied under the microscope and different chromosome structures and polymorphisms (karyotypes) characterized (*see* detailed description by Thorgaard and Disney 1990).

Cytogenetic studies of salmonids have demonstrated a large variation in the number of chromosomes depending on taxonomic status. Most strikingly, these studies show that the different species and families of salmonids have a very high amount of DNA per cell compared to almost all other fish species. Only two groups of fish, namely Salmonidae and Catostomidae, have, as an entire taxonomic group, such a high DNA content. This suggests that these groups have a common tetraploid ancestor. However, these "ancient tetraploids" now seem to be in a process of becoming diploids.

The actual number of chromosomes in the most important salmonid species varies within the genera (*see* Allendorf and Thorgaard 1984). For Atlantic salmon the number of chromosomes are 54-56 compared to 77-82 in brown trout (*Salmo trutta*). The different species of Pacific salmonids have a chromosome number of 53-54 in pink salmon (*Oncorhynchus gorbuscha*) up to 64-68 in cutthroat trout (*O. clarki*). The number of chromosomes seems to be somewhat higher in the *Salvelinus* genus. All species belonging to the Salmonidae family have about 80% as much DNA per cell as mammalian species and twice as much as other related fish species. Another feature is that the actual number of chromosome arms for all species of salmonids is approximately 100, again twice as high as in related fish. For comparison, species of the family Osmeridae have a chromosome number of 50, chromosome arms of 60 and the DNA content per cell is about 25% of that of mammals (Phillips and Ihssen 1990).

The existence of a large number of duplicated gene loci is also supported by a large number of isozymes found in different salmonid species. Some of these were discussed in detail by Allendorf and Thorgaard (1984) who gave examples of different enzyme systems found in salmonid species. The number of loci controlling a specific tissue enzyme may be estimated by electrophoresis and selective staining. Studies of protein loci in rainbow trout show that about 45% of the loci have duplicate expression, which suggests that approximately 50% of the ancestral, duplicated loci have no functional genes and are no longer detectable by their products.

The importance of gene duplication during the evolutionary process has been discussed in detail by various workers (e.g., Ohno 1970). Gene duplication usually involves a tandem duplication of a single gene locus or cluster of gene loci, or some linkage group at a given time. The process of polyploidy, on the other hand, represents a simultaneous duplication of every gene loci within the whole genome. Although naturally occurring polyploids can be found among individuals of most species, fish are usually diploid. As mentioned above, the family Salmonidae is one of the two exceptions which are known to be of tetraploid ancestral origin.

Polyploidy has for a long time been considered to be of importance in the evolutionary process. The success of the tetraploid salmonid fish species in adaptation to various environmental conditions and life histories is believed to be dependent on chromosome rearrangement and additions of genes with new functions. According to Ohno (1970), such gene duplications are essential for progressive evolution. One copy of the gene can be conserved for performance of the initial function while additional genetic raw material is provided for developing new enzyme systems with other metabolic properties. The success of salmonids living in different environments (fresh water, salt water) could be associated with the tetraploid origin and evolution of a complex set of enzymes controlling a large variety of biochemical processes.

Evolution of polyploidy in animals is believed to be very limited due to reproductive barriers. Establishment of a tetraploid strain requires fertile individuals of both sexes, but the existence of naturally occurring polyploid individuals and laboratory experiments have shown that this process is possible. New information concerning sex determination mechanisms in rainbow trout show that polyploidy does not influence the normal reproductive mechanism.

### *Homing to Spawning Sites and the Stock Concept*

One of the most fascinating features of salmonids is the tendency and ability to return to their home river and the sites where they were born. The homing phenomenon is treated in detail elsewhere and different homing mechanisms are discussed (*see* Chapter 3). The homing of salmonids is, however, basic for understanding of the genetic structure within a salmonid species, the stock concept, and different management and conservation strategies.

The homing tendency of Atlantic salmon was recognized several centuries ago. This specific behaviour of salmon was possibly well known to local people, and several priests (Hector Boece, Scotland; Peder Clauson Friis, Norway) gave a correct description of salmon behaviour in the 1600s (*see* Mills 1989).

Salmonid populations from different rivers or tributaries within large river systems are known to differ in a number of life history characters. These are believed to result from specific adaptation to local environments. This means that a river and lake system, in principle, can be inhabited by several sympatric populations of the same salmonid species. Natural selection, working over many generations, has selected the gene combinations optimal for survival in specific environments. This adaptation process is facilitated by the homing ability of salmonids resulting in the development of self-sustaining, local populations within salmonid species.

The stock concept in the management of salmonid populations has also been long acknowledged in North America. The early acceptance and use of the stock concept started in British Columbia more than 100 years ago (*see* McDonald 1981). Different species of Pacific salmon as well as different salmon runs within a river system were treated separately. In his comprehensive discussion of Pacific salmonid populations, Ricker (1972) referred to a conference on salmon problems in Ottawa, Canada, in 1938 where the term "stock" was introduced. This term should be used "to describe the fish spawning in a particular lake or stream (or portion of it) at a particular season, which fish to a substantial degree do not interbreed with any group spawning in a different place, or in the same place at a different season".

New methods developed in the 1960s and 1970s by population geneticists (*see* Allendorf and Utter 1979; Ferguson 1980) have been used to reveal the genetic population structure of salmonids. The relationship of the "stock" concept to "spawning units" and heritable characters to be transferred through the generations suggested a more precise definition based on genetic principles and concepts. This is clearly expressed in the proceedings of the 1980 "Stock Concept International Symposium" (STOCS) held in Ontario, Canada (*see* Can. J. Fish. Aquat. Sci. 38). One proposed definition (Booke 1981) for general application in fisheries management was expressed as "a stock is a species group, or population, of fish that maintains and sustains itself over time in a definable area". In more explicit genetic terms this means a Mendelian population, which shares a common gene pool and is in genetic equilibrium according to Castle-Hardy-Weinberg principles where gene frequencies are constant over generations. Another definition of "stock" proposed in one of the synthesis papers from this symposium (Ihssen et al. 1981) involves "an intra-specific group of randomly mating individuals with temporal or spatial integrity". In the identification and characterization of discrete stocks many biological parameters (physiological, behaviourial, morphological, meristic) and genetic characterizations (cytogenetics, biochemical genetic profiles) are used.

### *Quantitative Genetic Traits*

Quantitative genetic traits include characters that have a continuous variation among individuals measured. The length of fish in a population tends to follow a normal distribution where individuals cannot be grouped into a small number of discrete classes. The length difference between the individuals measured could be of any value within the range of the normal distribution.

Such traits are under the influence of both environmental factors and the genetic constitution of the individuals, and the phenotypic traits observed are the results of an interaction between the genes and a particular environment. Quantitative traits are usually under the control of many genes or gene complexes, and the degree to which the actual trait is influenced by genetic factors varies greatly from trait to trait. The need of a measure to estimate the genetic control of a quantitative trait is obvious and the most commonly used parameter is the *heritability*, symbolized by $h^2$. It is estimated by dividing the additive genetic variance for the trait by the total variance observed under given environmental conditions. Estimation of the genetic variance requires a controlled arrangement of matings among individuals of known relationship. The value of the heritability ranges from 0 to 1.0, with 1.0 indicating that the variability observed for the trait in question is completely determined by genetic factors. Most quantitative traits, however, usually have values in the range from 0 to 0.4 (*see* next section), which means that most characters are under influence of both genetic and environmental factors. The value of heritability is, however, not a specific characteristic of the trait itself, but of that trait in a particular group (population) in a specific environment. This implies that the same trait may have different heritabilities in different populations, under different environmental conditions.

In his comprehensive review on Pacific salmon, Ricker (1972) discussed morphological as well as a large number of life history characters for different stocks and species. As mentioned, the estimation of the genetic influence on a specific character in terms of the heritability has to be measured in designed experiments that are impossible to perform under strict natural conditions. A common approach is to compare the performance of groups of fish from different stocks under identical environmental conditions and ascribe any differences to their heredity. The influence of environmental conditions could be investigated by rearing the same groups of fish under controlled conditions in the laboratory or in different environments. The data discussed by Ricker (1972) were based on all species of Pacific salmon including morphologic and meristic as well as a number of important life history traits. Differences among stocks were found with respect to vertebrae number, fin ray, number of gill rakers, number of parr marks, number of scales, number of pyloric caeca and colour at maturity, which in most cases could be ascribed to be at least partially under genetic control.

With respect to life history traits, the evidence suggests that migration behaviour of fry, ocean growth and size at maturity, age at seaward migration, age at maturity, season of return and spawning time are partly determined by heredity and in most cases are stock specific (Gharrett and Smoker 1993*b*). The observed characters and the variation detected among individuals and stocks are results of a complex interaction between genetic and environmental factors determining the diversity detected among locally adapted populations of salmonids.

In the STOCS symposium proceedings, Saunders (1981) presented a list of stock-specific differences observed for 16 different characters in Atlantic salmon. This information has been updated recently and includes several of the same traits as Ricker (1972) discussed for Pacific salmon. During recent decades more attention has been paid to performance traits of importance in salmonid farming: fecundity, egg size, age at sexual maturity, growth rate and pattern, disease resistance and low pH tolerance. Such traits and the estimated heritabilities are dealt with in more detail in the selection on breeding (below). The information on these characters is based on on-going studies and therefore does not represent a complete account of all important population traits. As they are also influenced by environmental factors to a varying degree, it is likely that natural selection has played a major role in the evolution of the adaptive genetic differentiation within the different species and populations of salmonids.

### *Qualitative Traits*

In contrast to the continuous variation of quantitative genetic variation discussed above, qualitative genetic variation is present for a character when the individuals can be grouped in a discrete number of phenotypic classes. The phenotypic properties are entirely determined at specific regions on the DNA molecules (gene loci), and variation is due to alternative forms of the gene (termed alleles) in the population. In addition, the genetic variation seen is usually under control of a single gene or a limited set of genes.

Since Watson and Crick in 1956 presented evidence for DNA as the inheritance material and a model for its molecular structure, there has been an immense development in molecular biology and genetics. The linkage from the stored information as nucleotide sequences in the double-stranded DNA molecules (which constitute the main parts of the chromosomes) to the different proteins and enzymes, is carried out by the process of transcription. The main principle is that the nucleotide sequence constituting a specific gene specifies the sequence of amino acids in a specific protein, and thus the properties of the protein in question are determined. A phenotypic character in the protein, based on variation in molecular structure, therefore reflects a genotypic character in the genetic material of the individual.

Qualitative genetic variation is almost in all cases determined by the molecular structure (differences in nucleotide sequences) of the genome itself, but the phenotypic variation can be observed or identified on different levels and by a variety of methods. Some of these are briefly discussed below.

*Morphological Traits*. The pea experiments carried out by Mendel are classical examples of observable characters under control of single genes which follow a simple segregation model. In many fish species there are similar studies of morphological traits, especially of pigmentation or colour and scale characteristics (*see* Kirpichnikov 1981). The genetic basis for such morphological polymorphism must be documented in terms of parent-offspring relationships and, preferably, by breeding studies to reveal the detailed inheritance model of the trait.

In spite of the description of an enormous number of morphological and pigmentation/coloured phenotypes within most species of salmonids, there are relatively few cases where controlled crossing has actually determined the inheritance mechanism. The influences of environmental factors on the colour intensity and variation according to life history stages, have probably caused most workers to neglect the role of inheritance. Some clear-cut examples, however, are known in rainbow trout with respect to pigmentation. These include a complete albino phenotype, gold pigmentation and metallic blue pigmentation (*see* Kirpichnikov 1981 and references therein).

A morphological marker has recently been described in brown trout. In a mountain lake in Norway, a fine-spotted phenotype trout has been known among local people. In contrast to the common, large-spotted brown trout, this variant has small and dense spots on the main body and some atypical spots in the eyes (Skaala and Jørstad 1987). From breeding studies in the hatchery, the spotting pattern was found to be controlled by two codominant alleles at an autosomal locus (*see* Figure 1). This fish can be identified without sophisticated analytical methods and is ideal for use in experiments where a genetic marker is needed.

*Immunogenetic Methods*. The genetic control of the variation in blood groups of humans was early established and used in practical medicine as well as in anthropological studies. This variation followed simple Mendelian inheritance in all other species investigated and was highly polymorphic in natural populations.

These methods were among the first to be applied in population studies of fish. There were, however, several technical problems associated with stability of fish blood cells and especially with respect to reliable preservation of the isolated antisera. For these reasons (*see* Hodgins 1972) and because of the rapid development of enzyme electrophoresis, immunological methods have had very limited use in fish genetics during the last decade.

New developments in this field in recent years have solved some problems and opened several new possibilities. The immune response has been shown to be associated with the major histocompatibility complex consisting of closely linked genes which are highly variable. The earlier production of polyclonal antibodies has been replaced by developing cells which produce monoclonal antibodies and can be kept in culture for the time required. A specific program for developing monoclonal antibodies in fish is needed; these could be used in studies of the fish immune system and in detailed population genetics (Davis et al. 1990).

*Protein Electrophoresis*. Proteins in solution are charged molecules affected by the amino-acid composition and the pH in the buffer solution. Thus, changes in the

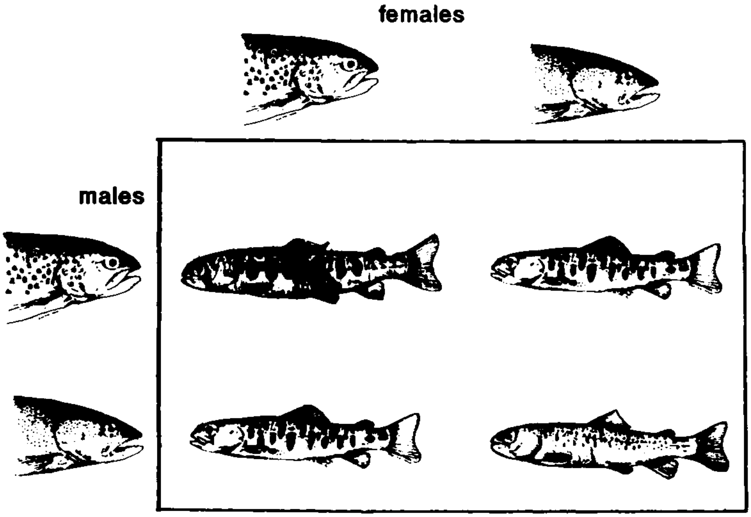

FIGURE 1. Example of Mendelian inheritance of the fine spotted trait in brown trout based on data from Skaala and Jørstad (1988). For detailed description, including colour pictures, see Skaala and Jørstad (1988) and Jørstad et al. (1991).

nucleotide sequences in the DNA may cause changes in the overall charge on a specific protein molecule. Like other charged particles and macromolecules in solution, they will move in an electrical field according to the magnitude and the direction of the field. This process, called electrophoresis, is able to separate molecules that differ in their net charges, and is a very powerful analytical tool in biochemistry. The principles of electrophoresis, use of stabilizing media, different staining techniques, interpretation of stained bands and application of the methods in various biological fields, have been described in detail in many textbooks and handbooks (*see* Harris and Hopkinson 1976; Richardson et al. 1986; Aebersold et al. 1987; Utter et al. 1987; May and Krueger 1990; Morizot and Schmidt 1990).

During the last decade, protein electrophoresis and specific staining of enzymes have been the most powerful techniques in studies of genetic variation in many species. The relatively simple equipment needed, efficiency of sample processing, and usually simple interpretation of the variation in terms of genetic models are the basic reasons for its successful use in population studies.

*Chromosomal Polymorphism.* As discussed in a previous section, the number of chromosomes varies among salmonid species. The number of chromosomes also varies to some degree within a given species and this variation seems to follow a geographic pattern. Most clearly, this kind of variation is seen in pink salmon and rainbow trout. For the latter species, the overall number of chromosomes varies between 58 and 64. Since pink salmon normally have a rigid two-year life cycle, the odd and even year sympatric spawning populations are reproductively isolated from each other. The fish spawning in even-numbered years have a chromosome number of 52, while the odd-numbered individuals have 53 or 54. Additional chromosomal variation (different karyotypes) can be detected among the odd-numbered populations, which then can be distinguished based on this polymorphism (Phillips and Ihssen 1990).

Chromosomal polymorphism has also been used for population discrimination in Atlantic salmon. There appears to be a difference in the chromosome number between European salmon and salmon from the western Atlantic, which have a chromosome number varying from 54 to 56, while salmon from east Atlantic have a number from 56 to 58. Some differences in the arm number have also been detected (Hartley 1987). Chromosome polymorphism has been used to distinguish between native and foreign stocks of Atlantic salmon in a Spanish river (Garcia-Vasquez et al. 1991).

The actual number of populations and individuals that have been karyotyped in salmonids is, however, quite low, mainly because of technical reasons. Reliable sampling must be based on dividing cells which limits the practical use of this technique in large-scale investigations. Development of more efficient and reliable techniques will be needed before these polymorphisms can be screened on a large scale.

*Restriction Fragment Analyses of Mitochondrial DNA.* The total DNA material in a single cell probably represents a coding capacity of 40,000 - 60,000 genes. Most of this DNA is located in the nucleus, but about 1% of total DNA is found in a small circular molecule in the mitochondria outside the nucleus (mt DNA). Extensive studies of mt DNA during the last few years have shown that this genetic material has probably evolved 5-10 times faster that nuclear DNA and therefore should demonstrate a large amount of genetic differentiation among populations and species. Mt DNA is under strict maternal inheritance, which offers the possibility of following maternal lineages within a species.

Practical analyses involve isolation of the DNA from fish tissue (Chapman and Brown 1990). The mt DNA is then digested with a restriction enzyme which cleaves the DNA molecule at a specific site recognized by the enzyme. There are many different classes of restriction enzymes commercially available; each one cleaves the DNA molecule at a site denoted by a specific nucleotide sequence. The digestion of DNA with restriction enzymes usually produces a varying number of DNA fragments that differ in size depending on the number and the localization of the restriction sites on the molecule. The fragments are separated on agarose gel by electrophoresis, and the band pattern is determined. The variation in different restriction band profiles (termed clones; DNA composites; fragment genotypes) are used to compare individuals, populations or species. A detailed description of the different techniques involved and applications in fisheries management are given by Ferris and Berg (1987).

The use of restriction enzymes to reveal variation in the mt DNA genome has recently been widely used for salmonids. In a recent review by Gyllensten and Wilson (1987), the principles of restriction fragment analyses of mt DNA in fish are discussed and several important results from a number of studies of salmonid populations are given. In more recent studies, even sympatric genetically distinct populations of anadromous and non-anadromous Atlantic salmon are revealed by mitochondrial DNA variation (Birt et al. 1991), and Hynes et al. (1989) showed that such variation could be used as genetic markers for brown trout.

This powerful analytical method (Bermingham 1990) is the ideal tool for certain studies. Although the number of available restriction enzymes is steadily increasing, the time required to sample, process and digest DNA is a present limitation to the technique. However, application of polymerase chain reaction and direct sequence analysis (*see* below) also offer possibilities for further studies on mitochondrial DNA as documented, among others, by McVeigh et al. (1991).

*DNA Fingerprint Analyses and Sequencing.* The revolution within molecular biological methods and especially within gene technology in recent years, has provided new approaches for use in genetic studies of populations (Whitmore et al. 1990). These include establishing nuclear DNA fragment profiles (fingerprinting) on all levels, allowing identification of individuals, families, population units and species.

The digestion of total DNA from the nucleus usually results in a large number of DNA fragments with a large variation in fragment size. These can be visualized on a agarose gel after electrophoresis by annealing to a radioactive DNA probe, which only binds to the fragments that have complementary nucleotide sequences to those of the probe. DNA isolated from an individual, digested with a restriction enzyme and probed after agarose electrophoresis, will produce a very complicated DNA fragment profile which constitutes the genetic "fingerprint" of that individual. Such individual DNA fingerprints are valuable in estimation of relationships between close relatives and in criminology, but are seen to be too complicated to use at the population level.

The development of single locus probes (slp) during the last years offers a new powerful technique for genetic studies in fish. This requires extracting DNA from a specific region on a multi-locus fingerprint gel which is indicative of single locus variation among individuals. The DNA is incorporated and cloned in bacteria, and different clones are isolated and screened for single locus variation. The identified single-locus DNA probes can be stored and produced for further use by simply keeping the bacteria in question.

Single locus probes were first produced and applied to study mating behaviour in birds. The same approach has in principle been used by Taggart and Ferguson (1990), who successfully developed several slps for salmonids. As shown in Figure 2 the fragment patterns are very simple and the methods seem very promising for use in a number of important fields of salmonid genetics. The enormous interest in using the new analytical tools and many different applications will probably cause a rapid development and refinement of the methods for large scale use in population studies. Variable minisatellite DNA single-locus probes for brown trout have been cloned by Prodøhl et al. (1994). Likewise Hartley and Davidson (1994) have studied distribution of genomic repeat sequences from Arctic charr (*Salvelinus alpinus*) among species of Pacific salmon and different charr species. Cutler et al. (1991) also used a polymorphism in the ribosomal RNA (rDNA) genes to distinguish Atlantic salmon from North America and Europe.

The smallest genetic differences that can be identified are single base-pair changes in the nucleotide sequence of the DNA molecule itself. Sequencing methods are presently well developed in biochemical research and can be carried out automatically in specially designed apparatus now available commercially. The problems are, however, connected to identification and isolation of the specific genes that are of interest.

In most cases the amount of the DNA material for a specific gene locus is very small compared to the total DNA in a cell. Therefore the purification of the gene locus in question must involve separation from the bulk DNA and extraction of sufficient amounts of DNA for sequencing analyses. The new advances in gene technology have provided knowledge and detailed information about replication and transcription of the DNA molecule. Of special importance are the DNA polymerases able to synthesize new DNA strands from a given DNA template (Clarke and Walsh 1993). One of the new advances utilizes the polymerase chain reaction (PCR) to produce large number of copies from a given gene locus. Given all the necessary enzymes and nucleotide precursors and a promotor to identify the gene locus in question, the PCR-reaction will synthesize a large number of copies of the gene. This reaction has been automated, and can be used to amplify the separate genes in quantities large enough for sequencing.

In such cases total DNA can be used, which is easier to extract than mt DNA. Since specific DNA regions or genes are amplified and sequenced, it is possible to carry out direct comparison with published information on the same genes across populations and species. In the case of allozyme electrophoresis and mt DNA fragment analyses, samples from different laboratories must be run side by side on the same gel to verify identity. At present, the PCR method has some limitations with respect to the number of samples that can be analyzed in a given time and the number of genes available for amplification. However, development in this field is very rapid and it is likely that a powerful tool will emerge with enormous potential for research in population genetics.

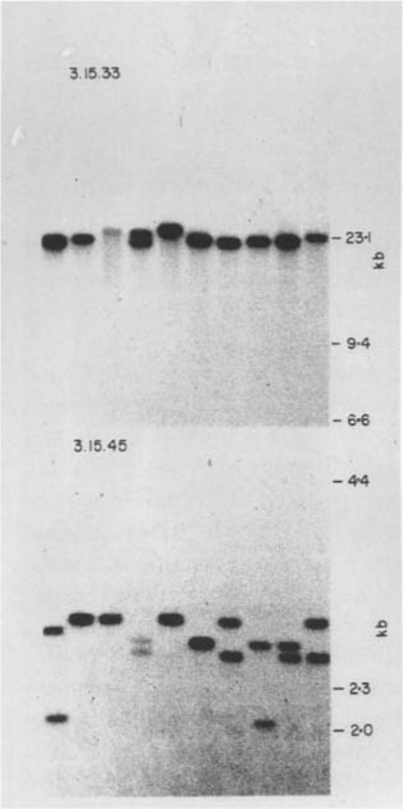

FIGURE 2. Single-locus minisatellite DNA variation in Atlantic salmon. The variation was obtained by using two different single-locus probes (3.15.33; 3.15.45) and have recently been shown to be in accordance with Mendelian inheritance (Taggart et al. 1995). (Modified from Taggart and Ferguson 1990).

### *Intraspecific Variation*

The salmonid group consists of a large number of different morphological forms and different life histories. The general biology and life histories of the most important salmonids are given in Chapters 2 and 3, and only a few specific characteristics will be mentioned here. As described in the Introduction, genetic variation exists on different levels. In principle, genetic differences are found between individuals within a specific group of fish as well as between groups of fish (populations), in these latter cases mainly as frequency differences of inherited characters.

The present population and genetic structures of salmonid species are results of long-term evolution and adaptation to various environmental conditions and life histories.

The tetraploid origin of salmonids is thought to augment precise adaptation to particular environments, and the homing behaviour of salmonids may lead to the formation of genetically separate populations or stocks within a species (and within a geographical region). Early observations of the general biology of salmonids suggested that each species consists of a large number of such populations or stocks. Differences, such as in body shape, colour, or body size at return to spawning sites, have long been noticed by commercial fishers, anglers and researchers. Such characters are, however, dependent on both genetic and environmental influences, and thus are only partly valid for stock rehabilitation.

During the last two decades, the new developments in population genetics have provided the variety of genetic methods discussed above. The methods and applications in fisheries management have been reviewed recently in the book edited by Ryman and Utter (1987). Based on new genetic techniques (*see* Whitmore et al. 1990) the distribution of genetic variation and the population structure within the different species have been achieved. The published work on genetic variation in wild salmonid populations is very large, and it is impossible to present a complete account here. Some selected examples considered to be typical are, however, discussed more in detail below.

*Atlantic Salmon.* Genetic studies to identify Atlantic salmon stocks in different geographical areas started about 25 years ago. The initial studies were based on heritable variation in blood proteins and have been extended by enzyme electrophoresis mainly during the last decade (*see* Mills 1989 and references therein). At present more than 300 populations have been examined for a large number of enzyme loci, and the results so far demonstrate genetic differentiation between salmon populations belonging to different continents, within geographic regions, and even within large river systems (Ståhl 1987). Genetic differentiation (in terms of genetic distance based on genetic variation in tissue enzymes) seems to increase with increasing geographic distance between the populations.

The different sets of genetic information (chromosome polymorphism, isozyme variation and mitochondrial and nuclear DNA studies) convincingly support the concept that the Atlantic salmon are divided into a large number of populations or stocks that are more or less reproductively isolated (Crozier and Moffett 1989). This is also true for populations having different life history strategies (anadromous; land-locked).

The genetic information for Atlantic salmon is based mainly on loci coding for different enzymes. In comparison with other salmonids species (e.g., brown trout) based on the same methods, the genetic variation in Atlantic salmon seems to be limited (Ryman 1983). However, more recent reports (Crozier and Moffett 1989; Verspoor 1994) show a higher degree of genetic divergence of protein coding loci, and especially among nonanadromous populations, the interpopulation variation was found to be high.

*Brown Trout.* This species is known for a very high level of variability with respect to morphology, behaviour and life history. Based on observed differences between geographical regions, a large number of species were initially described as a part of a polytypic species. Recently, population genetic methods applied in studies of brown trout populations throughout the species range revealed an extremely genetically diverse species. In his review on protein variation in brown trout, Ferguson (1989) compiled allozyme information from 412 samples from literature. These represent 70 loci of which 54% were found to be polymorphic within the sampling range.

The high level of observed heterozygosity (4.7%) and range of mean genetic distance between populations (0.026-0.15) demonstrated the great genetic diversity of brown trout. Considering the variation on the individual level, each fish was on average heterozygous at 16% of the loci analyzed, which shows that the brown trout is one of the most polymorphic vertebrates studied to date. In his comparison of genetic variation in different salmonid species, Ryman (1983) noted that about 80% of total genetic variation in brown trout was distributed between different populations, in contrast with 20% for Atlantic salmon, and in Spain, Garcia-Marin et al. (1991) found that 60% of the total genetic variability was due to differences between natural populations.

The genetic diversity of brown trout is also demonstrated by the existence of sympatric populations within the same water course or lake. Several examples are described in detail (Ferguson 1989; Ferguson and Taggart 1991 and references therein; Figure 3). For example, three genetically distinct populations are found in Loch Melvin in northwestern Ireland. This unique trout community, consisting of three trout morphotypes called gillaroo, sonaghen and ferox, have been studied by Ferguson's group in Belfast for over a decade. Allozyme frequency data show that the three types are genetically distinct with little interbreeding. The trout types also differ in a number of aspects such as feeding preference, growth rate and, perhaps most importantly, spawning behaviour and preferred spawning sites.

*Cutthroat Trout.* This salmonid species is found in the western intermountain regions of North America and the western coast of the continent from Mexico to Alaska. The wide geographic range, the extreme genetic variation and ecological differences among regions have suggested a polytypic species. A number of different subspecies have been described and genetic analyses using enzyme electrophoresis demonstrate large genetic differentiation at the population as well as the subspecies level (for discussion *see* Allendorf and Leary 1988).

*The Pacific Salmon.* The Pacific salmon species have been extensively studied (Utter et al. 1980). In the 1950s, multiple haemoglobins in chum salmon (*Oncorhynchus keta*) were discovered (Haskimoto and Matsuura 1959). The multiple haemoglobins of Salmonidae have since been thoroughly studied, and as a rule the variability found has been shown to be associated with development (Kock et al. 1964; Vanstone et al. 1964) although Fyhn and Withler (1991) described genetic polymorphism in haemoglobins of chinook salmon (*O. tshawytscha*). Genetic variation in several tissue enzymes has been described, of which only a few can be mentioned here; for instance, LDH and PGM in

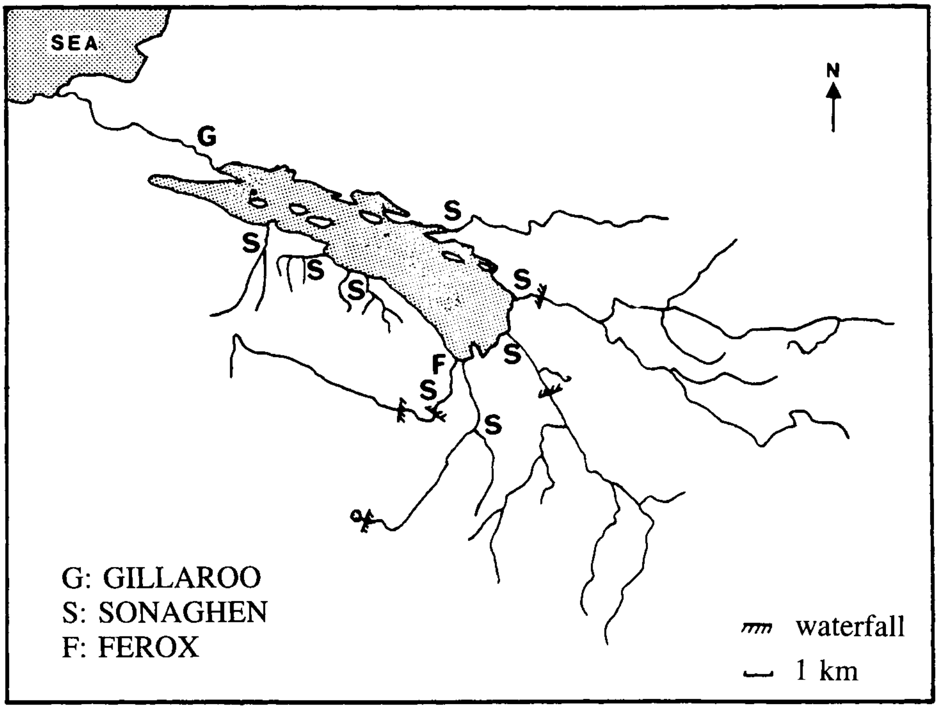

FIGURE 3. Intraspecific variation in brown trout in Loch Melvin, Ireland. G: Gillaroo — golden-brown or yellow-coloured body and fins with many large vivid or orange-red spots; spawn in river outlet. S: Sonaghen — light brown or silvery-coloured body with dark brown or black fins; many large black spots; spawn in all river inlets. F: Ferox — silvery or dull brown colouration sometimes with a slight greenish tinge; small, dark spots which could be absent; spawn in the largest river inlet. The populations are also very different when comparing several genetic characters (allozymes, mtDNA, minisatellite DNA). (After Ferguson and Taggart 1991).

sockeye salmon (*O. nerka*) (Hodgins et al. 1969; Utter and Hodgins 1970). Such traits have been used in a number of studies on population structure of *Oncorhynchus* species in the U.S. and Japan (for example, Okazaki 1978, 1979, 1981; Grant et al. 1980). Special attention is given to the extensive study conducted by Shaklee et al. (1991) on pink salmon which is a highly polymorphic species. Likewise, the application of polymorphisms in studies on the masu salmon (*O. masou*) complex in Japan (Nakajima and Fijio 1993) and the discussion on the dynamics and stability of genetic variation by Altukhov (1990) are notable. Use of biochemical polymorphisms in fisheries management with special reference to Pacific salmon have been presented and reviewed by Utter (1991); *see also* the discussion on genetic management on wild stocks on page 700. Recently, comprehensive investigations on genetic stock identification in Pacific salmonids were carried out for the purpose of mixed stock analysis (Shaklee and Phelps 1990; Shaklee et al. 1991). Results of these and other studies were presented in Juneau, Alaska in 1993 (Can. J. Fish. Aquat. Sci. 51[Suppl. 1]).

*The Charrs.* Polymorphism in serum esterase of Arctic charr was described by Nyman (1967), and used for a new approach to the taxonomy of what has been called the "*Salvelinus alpinus* species complex" (Nyman 1972). Later a vast literature on genetic variation of *Salvelinus* spp. has occurred, showing that these species are very variable both within and between populations. Such studies include the description of protein loci in *S. alpinus* by Anderson et al. (1983) and the experiments on joint segregation of biochemical loci conducted on brook trout (*S. fontinalis*) as well as hybrids between lake trout (*S. namaycush*) and brook trout (May et al. 1979). In brook trout, very high between-strain variations were found, suggesting subspecific differences between northern and southern brook trout populations in the U.S. (Stoneking et al. 1981).

## BREEDING PROGRAMS FOR FARMED FISH STOCKS

### *Important Criteria for Choice of Species*

The first step in a breeding program is choosing the species. For several reasons including history, value and public interest (reviewed in more detail below) the salmonids have been very widely used in many types of aquaculture. The species chosen for a breeding program should be based on many factors including the type of aquaculture to be done and the environmental parameters of the activity (Kanis et al. 1876; *see also* Chapter 4).

*Behaviour and Survival in Captivity.* One key criteria for species choice is that the species adapt well to the aquaculture conditions expected (e.g., high density rearing). A species that does fairly well under particular conditions will have a good chance of becoming domesticated after some generations under these conditions. The reproductive cycle must also be considered for intensive aquaculture in which the whole life cycle must be controlled.

Control of salmonid reproduction is now well established with incubation techniques established (*see* Chapters 1 and 5) and rearing techniques (*see* Chapters 6 and 9) still undergoing refinement. The large eggs (3-8 mm) and simultaneous development of numerous progeny facilitate the culture process. Several species of salmonids have been shown to adapt very well to conditions of captivity for the entire life cycle. This includes the rainbow trout, Atlantic salmon, coho (*Oncorhynchus kisutch*) and chinook salmon, and Arctic charr. Several other species have not been reared to date in this intensive manner, due to either biological problems or little or no effort.

*Availability of Proven Strains within the Species.* The population structure of several salmonid species is well known, while that of others is still obscure (*see* page 671). The productive traits, including survival under artificial conditions, vary among strains, probably because of inherent characteristics. To develop improved "strains" for intensive and extensive aquaculture, base populations should be chosen from several natural strains, and then tested for productive performance under the actual rearing regime. Knowledge about the natural performance of the strains greatly facilitates this process. Such knowledge is available for strains of many salmonid species, and has to some degree been utilized when starting breeding programs (*see* below). Likewise this is true to a high degree when selecting strains for repopulation of lakes where the natural populations have been destroyed due to environmental disturbances, for example, the extensive use of the Tunhovd brown trout in Norway and the Gullspång brown trout in Sweden (Ros 1981), which both have shown high survival combined with high growth rate when transferred to new environments.

*Growth Performance in Captivity.* For intensive aquaculture, a high growth rate is essential. High growth rate indicates a high feed utilization, rapid turnover in the production facilities and less money required for investment and operating costs. Sexual maturation during the production cycle is a serious drawback and should be avoided.

Several salmonid species have demonstrated a growth rate in captivity that makes them fit for intensive aquaculture. They include rainbow trout, Atlantic salmon, several Pacific salmons and Arctic charr. Maturation during the normal production cycle frequently becomes a problem for nearly all the species, especially in the production of large fish, such as those used for smoking and similar processing. Age (or size) at maturation varies considerably both within and among populations. Here, choice of strains, genetic selection within strains, and proper husbandry are essential for reduction of loss due to early maturation.

*Availability of Gametes.* Species chosen for aquaculture must be available for culture in reasonably high quantities. Although collection of fry and young fish from nature may be practised for some non-salmonids, normally it is necessary to maintain the fish through the whole life cycle including hatching and start-feeding. Thus a base population has to be established. This base population should ideally represent the species' gene pool, or at least the more valuable strains within the species ("valuable" is related to the purpose of the aquaculture activity, *see* below). To prevent future inbreeding and possible inbreeding depression, the base population should be reasonably large. For stocking purposes, it is also important to conserve the genetic variation within the stock(s) (Krueger et al. 1981). For intensive fish farming the base population(s) should contain presumptive highly productive strains and as much as possible of the variation in productive traits to be used in selective breeding programs. For intensive aquaculture it may be wise to establish large, self-sustaining, captive populations with a large amount of genetic variation so that future importation of genetic material will not be necessary as this involves the risk of parasite and disease introductions.

For most salmonids, availability of gametes has caused no major constraints on aquaculture activities. Thus most species of interest have been available both for intensive culture, for sea ranching and for enhancement. However, specific strains of potential interest may have become extinct or greatly reduced in numbers of wild spawners before they could be cultured (Ros 1981), and due to legal constraints, sampling of broodfish for intensive culture has been prevented or reduced. However, by and large, salmonids are suited for a broad range of aquaculture because of the availability of live material, and lack of gametes usually has little to do with the choice of species within the salmonid group.

*Market Appeal and Value.* Cultured fish are going to be sold more or less in the same market as wild-caught fish. Cost of feed, investment cost, labour, and other expenses demand a good product of high market value. Thus, species for aquaculture should be valuable fish and preferentially known by the market before the production starts. Use of a species not well known in the market will require much planning and marketing promotion, which is an added expense and which will take time (*see* Chapter 15).

Atlantic salmon as well as Pacific salmon are known in the market, and they obtain prices that have made them highly attractive for intensive aquaculture. There are no essential differences in quality between wild-caught and cultured salmon, and providing that quality is high, the market appeal and value should be expected to be high for farmed salmon.

Rainbow trout has been subjected to intensive production in great quantities. Here, pan-sized fish are raised, while "salmon-like" sizes (2-7 kg) may also easily be produced. The latter has only partly been accepted by the market, although it can hardly be distinguished from salmon. Nevertheless rainbow trout is and will probably be one of the most useful species for aquaculture, especially because it can provide different kinds of products, and may be produced both in fresh and salt water.

Other salmonids have not yet become important for intensive aquaculture. Brown trout (sea trout), for example, is known by the market and obtains a reasonably high price, but moderate to low growth rates to prevent this species from becoming a candidate for intensive aquaculture. Its value for sport fisheries, however, make it an important species for enhancement and possibly for sea ranching. Arctic charr is less well known, and in spite of its suitability for production in artificial systems, the production of Arctic charr (and other charr species) by intensive aquaculture is still at a low level. Due to its tendency to overpopulate its environment, this species has little value for enhancement, although it has potential for sea ranching in areas usually subarctic where anadromous strains are found.

By and large, most species of *Salmo*, *Oncorhynchus* and possibly *Salvelinus* are suited for some type of aquaculture. For intensive aquaculture, however, several species display traits that restrict their potential. At the moment rainbow trout, Atlantic salmon, Arctic charr and possibly chum, sockeye, chinook and coho salmon are the most important species for intensive aquaculture.

## EVALUATION AND SELECTION OF STRAINS FOR BROODSTOCK

### *Wild Strain Evaluation*

*Traits to be Considered and Genetic Mechanisms.* When evaluating wild strains for any type of aquaculture, one must consider their potential performance under aquaculture conditions. The purpose of the aquaculture activity will dictate which traits should be emphasized. Survival and adaptation to specific natural conditions will be of crucial importance for enhancement, but for intensive farming, growth rate and age at maturity will be very important. However, pre-evaluations of wild strains (or species) are only the very first step in a successful culture process. Complete evaluation can only be attained by trials of the species and strains under particular culture conditions. This is also the only reliable way of estimating the genetic component of observed variation (i.e., heritabilities, *see* below).

*1) Growth rates.* The importance of high growth rate under intensive farming conditions is obvious, and may also be significant for sea ranching. When enhancement is used to supplement sport fisheries, high growth rate is again desirable, as long as it has no antagonistic effects on other important traits such as survival. Genetic variation between strains has been reported for several salmonid species: Atlantic salmon (Gunnes and Gjedrem 1978; Nævdal et al. 1978*a*), rainbow trout (Gall and Gross 1978; Klupp et al. 1978; Ayles and Baker 1983; Linder et al. 1983), brook trout (Cooper 1961), and Arctic charr (Berg 1989). However, Gunnes and Gjedrem (1978) reported that the variation among families within strains was nearly as great as the variation between strains for some species. Within-strain selection for growth may be as important as the choice of salmonid.

Due to the difficulty in measuring food consumption on a sufficiently large scale, it is not known whether variability in growth rate is caused by differences in food intake or conversion efficiency. Kinghorn (1983*b*) found that only a small part of the variation in growth rate of rainbow trout was caused by food conversion efficiency, while Hershberger (1978) found significant differences between stocks and stock crosses of coho salmon in food conversion efficiency based upon measurement of growth increment and food actually eaten by the fish. Food conversion efficiency may well become more important in selection programs as a means to reduce the cost of production. If so, methods for determining the conversion efficiency of individuals grown in mass culture will be required.

2) *Age at maturity*. Salmonid age at maturity is of great significance in intensive culture as well as in sea ranching or extensive farming. Maturation stops somatic growth, reduces flesh quality, and even causes increased mortality in intensive culture as well as in nature. Maturation during the normal production cycle thus is very costly.

Strain differences of Atlantic salmon in age at maturity are well known (Dahl 1916; Berg 1964; Scarnecchia 1983; Saunders 1986). Variability in the age at maturity under natural conditions seems to be reflected when wild offspring are cultured (Gunnes and Gjedrem 1978; Nævdal et al. 1978*a*; Glebe et al. 1979; Nævdal 1983; Gjerde and Refstie 1984; Glebe and Saunders 1986). However, age at maturity is also directly influenced by environmental and life history factors. Scarnecchia (1983), in his extensive study of Icelandic salmon stocks, concluded that age at maturity is not a direct causal response to any physical environmental factor, and can be best understood with reference to the entire life history pattern of each stock.

Also in Pacific salmon species and rainbow trout, extensive interspecific and intraspecific variation in age at maturity are found (reviewed by Healey 1986 and *see* Gall et al. 1988). Pink salmon, on one extreme, always mature in their second year of life, where sockeye salmon possess 22 mature age categories when the various combinations of fresh water and marine residence are taken into account. Among charrs (Berg 1989) and brown trout, considerable strain differences in age at first maturity are also found.

3) *Survivability*. Survivability is a complex trait, and variation in survival is common and easily observed. To identify inherent factors for this trait, one needs to separate the possible causes of variation and try to analyse them individually. It is often difficult to distinguish environmental and genetic effects on survival. Environmental effects are often strong and occasional, and can easily mask genetic variation. Experiments intended to reveal the causes of variation in survival rate must be very well planned to permit correct conclusions.

Resistance against diseases and parasites is often a very important factor in survival. There is now a growing literature on genetic variation in disease resistance (Winter et al. 1980). However, many studies fail to distinguish inter- and intrastrain variation. One of the first examples of interstrain variation in disease resistance was described by Gjedrem and Aulstad (1974). They found that Baltic Atlantic salmon were considerably more vulnerable to vibrio disease than were Atlantic salmon originating from Norwegian rivers. This phenomenon was explained as an adaptation based upon the chance of being infected by saithe, *Polachius vireus*, a main host for *Vibrio anguillarum*, which is absent from the Baltic. Heritabilities estimated at an intrastrain level were comparatively low ($h^2 \approx 0.1$).

Chevassus and Dorson (1990) reviewed the literature concerning the genetics of resistance to disease in fishes. Variation among strains of several salmonid species have been found for both viral and bacterial diseases. Heritability estimates are very variable, but, in many cases, high enough for strain improvement through a quantitative approach. Fevolden et al. (1991) selected lines in Atlantic salmon and rainbow trout for high and low cortisol response, and they found evidence for a genetic component to the stress response in both species when the $F_1$ generation was exposed to standardized stressors. The objective was to obtain indirect measures for disease resistance. Gjedrem et al. (1991) reported high heritability estimates (*see* page 686 for mortality due to furunculosis) indicating that resistance may be effectively improved by selective breeding. An overview of different methods and possibilities for genetic improvement of disease resistance in salmonids were given by Fjalestad et al. (1993). Another possibility discussed by Chevassus and Dorson (1990) is genetic improvement through chromosome engineering and gene technology (*see* Chapter 17).

Host-parasite interaction is another important trait. In an aquaculture environment one expects to have at least the same parasites (and diseases) that exist in the local area. Endemic strains should be adapted to live with their disease agents, and thus may be safer in the hatchery than non-adapted foreign strains. By the same logic, parasites (and diseases) accompanying introduced fish may create disasters among local fish strains. One serious example is the transfer of the fluke *Gyrodactylus salaris* by Baltic salmon into Norwegian Atlantic salmon hatcheries, and from there, by accident, to Norwegian rivers (Johnsen 1988). This clearly shows interstrain variability (adaptation) in the host-parasite relationship. Although the Baltic Atlantic salmon stocks tolerate the parasitic fluke (Bakke 1991), its introduction into Norwegian rivers nearly exterminated the natural stocks of Atlantic salmon in infected rivers (Johnsen and Jensen 1986). This example clearly demonstrates the importance of being careful when transferring strains among geographical areas, to avoid creating new host-parasite interactions.

Egg quality is very variable in cultured brood stocks, but there is little evidence of a genetic component to the variation. Instead, differences mainly appear to be connected to the nutrition and handling of broodfish. However, significant differences in egg size have been reported between strains for several salmonid species (reviewed by Gjerde 1986). Medium to high heritability estimates have been reported for this trait, as well as for other traits connected to reproduction (egg volume, egg number, *see* Gjerde 1986). The practical value of these traits in a hatchery situation is not very clear, although Gall (1974) reported positive correlations in rainbow trout between egg size and hatching rate. Springate and Bromage (1985), Chevassus and Blanc (1979) and Gall (1974) have reported positive correlations between egg size and growth rate in the period from hatching to up to 75 days of fry age. This is possibly due to maternal effects.

Return rate of sea-ranched salmonids is a very important trait in enhancement programs. This trait is connected to migratory behaviour, ability to home to the natal river and survival in the sea. Strain differences in migratory behaviour are discussed by Saunders and Bailey (1980). Stabell (1984) reviewed the literature concerning homeward migration of salmonids and suggested that inherent olfactorial recognition of strain-specific pheromones (or possibly imprinting of the smolt on such pheromones) is responsible for homing. This theory is refuted by several authors, as reviewed by Hansen et al. (1987).

Acid-water tolerance may be an important factor both in hatchery rearing and in survival of natural strains under changing environmental conditions. In brown trout inter- and intrastrain differences in tolerance to acid water has been observed (Gjedrem 1976*a*; Edwards and Hjeldnes 1977), and such variation has also been documented in Atlantic salmon and brook trout (Robinson et al. 1976; Schom 1986).

Salinity tolerance or degree of anadromy is an important factor for intensive saltwater aquaculture as well as for sea ranching. Delabio et al. (1990) found significant differences in growth and survival between two strains of Arctic charr reared in seawater under similar conditions. Seawater tolerance is temperature dependent in Arctic charr (Wandsvik and Jobling 1982) and probably also in other salmonids. Norwegian fish farmers have recorded high and variable levels of rainbow trout mortality in seawater during winter, possibly reflecting different degrees of anadromy among the original, natural strains.

Unknown causes, however, account for much of the variation in survival reported in the literature. Interstrain survival differences in culture probably reflect strain adaptation to different natural environments, some of which are more similar than others to the culture environment. Iwama et al. (1992) found clear strain-specific differences in the stress response in juvenile coho salmon. Thus, to maximize survival, strains should be chosen for aquaculture on the basis of similar natural environmental conditions (e.g., temperature, water quality) to those which may be experienced in the culture environment.

Gjedrem (1983) gives an overview of the literature on variability in survival. He also discuss the difficulties in interpreting such data, and provides estimates of heritabilities. Generally these estimates are low on an interstrain basis. However, Robinson and Luempert (1984) estimated moderate to high heritabilities for survival for different life history stages of brook trout.

4) *Other traits*. Stock-specific responses to the environmental cues for smolting and for triggering migratory behaviour possibly exist (Saunders and Bailey 1980). For example, smolts in headwaters of long rivers start their downward migration earlier in spring (perhaps even in the preceding autumn) than do smolts in short rivers in order to reach the estuary when seawater conditions are suitable.

Saunders and Bailey (1980) also discussed seasonal run patterns which almost surely have a genetic basis in Atlantic as well as in Pacific salmon. It is also well known that spawning time differs between strains of Atlantic salmon, brown trout, rainbow trout, and Arctic charr (Berg 1964), and that the interstrain variability has a genetic basis because the differences persist when the strains are raised under aquaculture conditions. Spawning patterns (one or multiple spawning) also seems to be an Atlantic salmon strain characteristic (Saunders and Bailey 1980).

Genetic variation in flesh pigmentation in Atlantic salmon (Torrissen and Nævdal 1988) and rainbow trout (Torrissen and Nævdal 1984) has been observed. However, as these observations were made on cultured populations, the variation could not be partitioned among and within strains. Chinook and coho salmon also display genetic control of flesh colour with strains having either white flesh or red flesh, or a mixture of both (Withler and Beacham 1994).

Other, more specialized strain characteristics are well known among several species of salmonids. Usually people know "their" strain of salmon and trout, although not whether the distinctive characteristics are environmentally or genetically controlled. Examples are the salmon strain in rivers of northern Norway (Berg 1964) and the salmon and trout strains described by Ros (1981) from the province of Värmland in Sweden.

*Types of Mating Programs.* The type of mating program to be chosen in wild strain evaluation depends on the goal of the program, type of information sought and the capacity of available rearing facilities. For intensive aquaculture it is important to design a mating program in order to estimate genetic parameters. The mating program should also maximize the genetic variation in the founder population; i.e., make the basic populations suitable for selective breeding in later generations, and avoid inbreeding (unless studies on inbreeding are a part of the research program: *see* page 691).

*1) Diallel crosses.* This design may be applied at both an inter- and intrastrain level. Egg groups from individual females within a strain or from groups of females from various strains are divided into batches and fertilized with different males or milt pooled from groups of males from different strains. On an intrastrain level one obtains crossing sets (2 × 2, 3 × 3), in which each group consists of full sibs which are paternal or maternal half sibs to other groups in the same set. Likewise, on a strain basis the total material consists of groups of pure strains and strain hybrids.

The diallel design is suitable for estimating sire and dam components of heritability, and also for estimating non-additive genetic variation, which will show up as "interaction" in data analyses. The major difficulty with this design is caused by asynchronous maturation among individuals or especially among strains. Stripping of gametes which are immature or overripe, causing fertility differences among the groups, make the results obtained difficult to evaluate.

*2) Family group separations, hierarchal design.* In this design one male is mated to two (or more) females from the same or other strains depending on the purpose of the study. Such matings produce full-sib progeny groups which are paternal half sibs to other progeny groups. Such designs are well suited for heritability estimates, especially based on sire variance component because it is unaffected by non-additive effects.

*3) Other mating programs.* Mass mating (*see* page 689), with or without phenotypic selection, may be practised to reproduce wild strains for aquaculture. Mass selection here simply means that the "best" parent fish, judged from a superficial examination, are used for broodstock. To evaluate the effect of such a mating program, an unselected control population is needed, which may be obtained by fertilizing an egg batch consisting of small quantities of eggs from all females available with a milt batch pooled from all available males. Alternatively, progeny groups may be made by making individual crosses of one female and one male. Such strategies may be used for estimating genetic parameters, but the estimates may be biased because non-additive variation as well as systematic environmental effects (i.e., tank effects) may contribute to overestimation of the obtained values. If inbred groups are wanted, progeny groups resulting from brother × sister mating will give maximum inbreeding per generation (except for gynogenesis).

When crossing strains, one generation of random sib mating of the hybrid strains may be used to disrupt linkage units. Such practice may or may not be combined with mass selection for desirable traits.

*Sampling the Population.*

*1) Size and nature of sampling, determinants.* The aim of sampling populations for broodstock must be well defined before the work starts. Guidelines for the culture of fish stocks for resource management are given by Hynes et al. (1981). They stress that in establishing broodstocks to maintain anadromous runs of salmonids, random sampling to obtain founding parents is essential. To avoid genetic drift and inbreeding the minimum number of parents should be 50 of both sexes. This number is merely a "rule of thumb", and for more detailed examination of the risk of genetic drift and inbreeding, *see* Ihssen (1976). This model also may be applied to intensive aquaculture when random mating and, later, mass selection are going to be applied.

However, for intensive aquaculture, representative sampling of each wild strain is not essential. Instead it is more important to sample several strains to establish base populations for estimating genetic parameters, constructing selection indices and carrying out selective breeding over several generations. How much emphasis should be put on sampling each strain and on numbers of strains sampled depends upon the breeding goals and on the distribution of the genetic variation in the actual productive trait within and between strains. The performance of each strain in nature may give some, but incomplete, information on the performance in culture.

Sampling is also highly dependent upon the choice of mating program. Within-strain diallel crosses and hierarchal crosses require large rearing facilities. Until the offspring can be tagged, each family has to be reared separately, and thus the available facilities usually limits the extent of sampling. For sampling of several strains, and application of mating programs based on family groups, it will be possible to include only a few broodfish from each strain. These fish may be preselected based on information on their individual performance (for example size at age) in relation to the breeding goals, but they are not required to represent the gene pool of their strain. Although suitable for intensive fish farming, they are not suitable for management purposes even in the first generation of offspring. This is even more important in later generations when intentional and unintentional genetic changes are presumed to have taken place (*see* Hynes et al. 1981 for further discussion).

*2) Problems encountered.* There may become large discrepancies between wishes and possibilities when sampling wild strains. Especially with Atlantic salmon, the numbers of wild broodfish in the rivers are often very low, and less than are needed for maximum natural recruitment of smolts in the rivers. Especially multi-sea-winter salmon, presumably the most valuable for a foundation of selected strains for aquaculture, will not be available in sufficient numbers for representative sampling of the populations. In addition conflicts of interest will exist or arise because intensive fish farming and management of wild strains are represented by different groups of people, and management agencies in such situations make cooperation or sampling of natural strains difficult or impossible. For species and strains that exist in greater abundance, such difficulties do not exist, but such strains often are less valuable for aquaculture.

For reasons of capacity of the hatchery and fish-producing units, one has to determine how much emphasis should be put on each strain compared to the number of strains to be evaluated. The solution to this problem depends on the aim of the study, the existing knowledge of the performance of the strains, the distribution of variation within and between strains, and the availability of broodfish or gametes. As pointed out elsewhere, representative sampling of the strains is not necessary for development of strains for intensive fish farming, nor for development of strains for sea ranching where reproduction is assured in each generation. However, for complete evaluation of the performance of individual strains, with the aim of strain conservation or development of strains for restocking in empty watercourses, representative sampling requires the minimum number recommended for avoidance of inbreeding, i.e., 50 individuals of each sex. This demand, however, may be very difficult to attain.

*3) Case histories — wild strain evaluation, mating programs.* Hershberger (1978) described an experiment for evaluating life history performance of three strains of coho salmon. Gametes from 30 males and 30 females were collected at each location simultaneously, and inter- and intrastrain crosses were made based on pooled material from each location. This factorial design allowed a comparison of the pure-line population crosses with all possible combinations of interpopulation crosses in the same experiment. However, only a limited number of strains could be tested, because for making interstrain crosses, concurrent spawning time was a prerequisite.

The base populations for Norwegian selective breeding experiments with Atlantic salmon were mainly collected from natural strains all over the country (Nævdal et al. 1978*b*; Refstie and Steine 1978). This sampling procedure was a result of the poor availability of broodfish within strains and the available facilities at the rearing stations. (Each family group was kept isolated until tagged as smolt or presmolt). However, several strains were tested, (although variation within strains could not be evaluated in this way). The latter problem was partly overcome by sampling the same strains in two or more successive years. After one generation individual fish were crossed on the basis of individual and family performance regardless of strain of origin. However, several strains were not represented in later crosses because they did not appear to be valuable for intensive fish farming due to inferior growth capacity or early maturation.

At the NASRC a Salmon Genetic Research Program (SGRP) was established, mainly for the purpose of developing improved strains for sea ranching (Anon. 1981). This genetic research program included selection for four separate strains by introgression from several wild salmon stocks, and subsequent index selection among the hybrid progeny. Two strains were developed with grilse parents and two with two-winter salmon as parents. To accomplish this, the highest possible number of parents from a limited number of natural strains were sampled and crossed using a diallel or hierarchal mating design. To disrupt linkage units, one generation was used for random sib mating within the hybrid crosses, and the resulting strains were then used for index selection for traits favourable for sea ranching and cage culture.

*Determination of Genetic Information for the Strains Investigated.*

*1) Genetic mechanisms operating on traits of interest, phenotypic and genotypic correlations.* For planning of genetic breeding programs estimates of genetic parameters are essential. This is especially important when more than one trait is to be improved by selection (or by other methods), and when selection indices are going to be constructed. Such indices will depend on the relative economic importance of the traits in question and their mode of inheritance (i.e., heritabilities, *see* below), but also on the degree of covariation between the traits. Such covariation may be caused by pleiotropic gene effects or closely linked genes, by common environmental factors, or all of these. Covariation caused by genetic factors is measured by genetic correlation factors or genetic covariance, and corresponding phenotypic covariation is measured by phenotypic correlation or phenotypic covariance. These parameters may be estimated by simultaneous measurements of data for the actual traits on the same material; *see* Falconer (1960) for further discussion and methods of estimation.

Gjerde and Gjedrem (1984) estimated genetic and phenotypic parameters for several carcass traits of Atlantic salmon (within strains) and rainbow trout. The most interesting results concerning covariation were the positive correlations between growth parameters and maturity (measured as incidence level of 50% mature) indicating that selection for high growth rate ultimately will be followed by maturation at a earlier age. However, as also pointed out by Gjerde (1986), such results are difficult to interpret because several months before spawning, sex hormones begin to stimulate growth rate in maturing fish.

*2) Parental effects.* From the point of view of breeding, it is interesting to know whether the separate strains retain certain characteristics when bred in a culture situation. For Atlantic salmon this seems clearly to be the case (Nævdal et al. 1978*b*; Refstie and Steine 1978; Saunders and Schom 1985). Grilse parents largely produce grilse, while multi-sea-winter salmon largely produce large fish. For anadromous strains the performance in culture largely reflects performance in nature. However, for non-anadromous fish (brown trout, charrs), this seems not always to be true, possibly because in a crowded environment the fish are not able to realise their growth potential so that the development rates are hampered. Age at smoltification, which varies considerably in nature and may be shortened down to a few months by manipulation with environmental factors (*see* Chapter 8), is seemingly independent of the strain performance in nature.

*3) Heritabilities.* A vast amount of literature exists on heritability estimates of productive traits in salmonids. Productive traits mean all traits that are of importance to the goals of the culture activities, including reproductive traits. Heritability ($h^2$) is defined as the proportion of the phenotypic variance caused by additive genes, thereby excluding genetic variance controlled by dominance and epistasis (non-additive genetic variance). The heritability estimates, however, depend on methods used, and the different methods usually include some non-additive variations (*see* Falconer 1960 for further discussion). Heritability estimates based on full-sib pedigree groups usually exaggerate the effect of non-additive genes. Gjedrem (1983) and Gjerde (1986) reviewed the literature concerning heritability estimates in fish and shellfish. In general, heritability of body weight is moderate to high for all species studied, while age at maturity is variable, but occasionally high. Traits connected with reproduction (egg number, egg size, egg volume) also show high heritability estimates, while heritability of survival usually is low. However, Robinson and Luempert (1984) found moderate to high heritabilities for survival of brook trout. Attention should also be paid to recent results obtain by Gjedrem et al. (1991) into estimated heritability factors as high as 0.48 and 0.32 for sire and dam components respectively for mortality due to furunculosis.

As a rule, heritability estimates are made on a within-strain basis. However, interaction between strains and culture conditions for the different productive traits is poorly covered in the literature, but may well be responsible for some of the discrepancies reported concerning heritability estimates. Possibly most estimates based on sib groups also are overestimates because of non-additive genetic factors, and as a rule realized heritabilities (measured as responses to selection) are lower than estimates based on sib groups (Gjerde 1986). For the trait, age at maturity, and very complicated covariations between additive and non-additive genetic factors, as well as environmental factors may be the reasons for the occasional occurrence of high proportions of grilse experienced in fish farming in spite of several generations of selection on individual and family bases.

*4) Relative value of the strain.* At present it appears impossible to draw general conclusions about the relative value of particular strains in a culture situation. Some information may be obtained from knowledge of the strain's performance in nature, thus making pre-selection of strains possible. However, the strains must be assessed in the culture situation where they will be used, for example in intensive fish farming or sea ranching. This evaluation will also reveal variations to be used in selective breeding programs. Preselection of strains probably is most important for excluding strains not suitable for culture; for instance, strains with a high incidence of grilse are of minor value for fish farming.

### *Choice of Breeding Program*

The aim of a breeding program is to obtain cultured stocks or strains that give higher output in terms of money and resources. Genetic improvement is often referred to as *genetic gain.* Gjedrem (1975, 1976) discussed the possibilities of genetic gain in salmonids based upon the equation, cited from Falconer (1960):

$$\Delta G = i \times h^2 \sigma_p / L \qquad (1)$$

or

$$\Delta G = Sh^2 / L \qquad (2)$$

where $\Delta G$ = expected gain per year; $S$ = selection differential (difference between population mean and mean of selected broodfish); $\sigma_p$ = standard deviation in parent populations; $i$ = selection intensity or $i = S / \sigma_p$; $h^2$ = heritability (proportion of total phenotypic variance controlled by additive genes); and $L$ = generation interval.

On the basis of this formula, the possibility for genetic gain in fish is high due to the high phenotypic variation observed, relatively high estimates of heritability for productive traits, and the high fecundity of fish, which enables the application of strong selection. Due to the high fecundity, selected material may be distributed for commercial culture in each generation without disturbing the selection program. Thus, the cost of the program may be recovered after as little as one generation of selection. On the other hand the breeding program of any considerable dimension has to be planned very well because:

(1) breeding goals must be defined in accordance with market demand and available resources;
(2) usually more than one trait must be selected for, thus making constructions of selection indices desirable. Selection indices require heritability estimates and genetic and phenotypic correlations for each trait in question and an evaluation of the relative economic values of the traits; and
(3) inbreeding should be prevented. The effect of inbreeding is somewhat obscure, but it seems clear that at least hatchability, survival and growth rate are influenced by inbreeding (Gjedrem 1976*b*), and the high frequencies of abnormal phenotypes often observed in hatcheries is also possibly an inbreeding effect.

Thus, choice of a breeding plan or a breeding program is very important both for the cost/benefit ratio of the expected outcome of the program, and also for the best use of available facilities. Gall (1990) summarized the basic theory underlying the development of breeding plans, and the discussion below is based partly upon his considerations.

*Simple Programs to Prevent Deleterious Selection.* In nature most species and populations show a high degree of genetic variability. The biological meaning of this is not always clear, although it has been presumed that the genetic variance is very important for the population's adaptation to a changing environment. It is still not known whether this high degree of variability is needed in a culture situation. However, as long as this is unclear, it is best to take a conservative attitude and maintain as much as possible of the genetic variation.

In a breeding program deleterious selection may be unintentionally carried out in several ways. Risk of loss of genetic variation by inbreeding is a significant problem. In the case where males and females are mated at random, the rise in inbreeding coefficient, F, is given by the relationship:

$$\Delta F = 1 / 2N \tag{3}$$

Thus the effective number of the population is of crucial importance to prevent inbreeding depression and loss of genetic variability. As a general guideline, 50 individuals of each sex should be used for broodstock in each generation to keep increases of $\Delta F$ at a minimum. For more thorough discussion, *see* Ihssen (1976) and Hynes et al. (1981).

Correlation between traits may cause serious unintentional selection effects. Most conspicuous is the phenotypic and genotypic negative correlation observed between growth rate and mean age at first maturation (Gjerde 1986; Tofteberg and Hansen 1986). Although not very clear, such a correlation may result in a higher proportion of grilse in strains selected for high growth rate. However, because this correlation is not very strong, the problem can be avoided by careful planning of the selection process.

More serious is the practice sometimes seen in practical fish farming of using early maturing Atlantic salmon (grilse) for broodstock. As long as egg-shortage was a limiting factor in Norwegian fish farming, this practice was not uncommon, and probably resulted in selection for lower mean age at maturation or higher proportion of grilse among the offspring. Such effects are easily eliminated by selecting the broodfish from the late-maturing population.

The culture process by itself causes a semi-natural selection or domestication, which is an adaptation to life in the culture situation. For intensive fish farming, domestication is beneficial because the domesticated fish are better adapted to the culture environment and thus may express their growth potential better than "wild" fish. However, for use in semiculture (enhancement and sea ranching), domesticated fish may be detrimental because they are less well adapted to life in nature. Thus domestication should be avoided for such fish, and this may be done by using wild fish as broodstock for each generation. Use of broodfish from intensive fish farming for raising fingerlings or smolts for release into natural environments should be avoided unless it is shown that resulting domestication has no unwanted effects.

To avoid unintentional effects of selection one must monitor fish performance. When this is properly carried out, negative effects of genetic drift and inadvertent selection will not be difficult to avoid. Because it may be beneficial to sample wild fish to broaden the genetic variation of cultured fish, the fish farming industry should also support the maintenance of "healthy" wild populations.

*Mass selection.* Mass selection simply means that individuals that perform best under culture conditions are selected as broodstock for the next generation. The effect (genetic gain) of this procedure depends on the heritability of the trait in question and the strength of selection. Expected gain per generation may be visualized by Equation (4)(Gjedrem 1976*b*).

If the heritability is high, then the genetic gain obtained by mass selection will be considerable, and close to what would be obtained by more sophisticated and expensive methods. By comparing expected gain and actual gain reached by mass selection, the "realized heritability" may be obtained. Because of the time lag between generations, expected as well as realized gain should be measured as deviation (for example in percent) from the mean. This requires a control population, which may be obtained by breeding a random sample of the original population for comparison with the offspring of the selected parents.

As noted above, to avoid inbreeding and to keep the genetic variation high for traits other than those selected for, the number of effective parents should be at least as high as recommended above to avoid inbreeding, i.e., minimum of 50 individuals of each sex. However, when performing mass selection for several generations, inbreeding is difficult to avoid, as one unintentionally will select members of a few families because of their high performance. If individual families are not kept track of, there is a strong chance of only a few families being represented in the selected broodfish population. This will result in a high, but unknown, degree of inbreeding that will likely be reflected in inbreeding depression. The high frequencies of "cripples" and "monsters" often observed in closed-cultured populations may well be the result of such unintentional inbreeding.

*Family Selection.* In a narrow sense family selection refers to selection methods in which family groups (full-sib or half-sib families) are ranked according to their mean performance, and where whole families are saved or discarded. The advantages and limitations of family selection in its narrow sense are discussed by Gall (1990). He concluded that this method is advantageous over individual selection only when the correlation between family members (intraclass correlation) is low, indicating a low heritability, and the available rearing facilities permit raising of a high number of separate families.

However, for sex-linked traits and for traits that require slaughter of the individuals being evaluated, selection based on mean sib performance is an alternative method. As also discussed by Gall (1990), this method is advantageous compared to individual selection when the intraclass correlation is low and the numbers within families are high. In addition breeding values of traits like flesh pigmentation and fat content necessarily have to be measured by sib performance.

The most powerful tool for obtaining genetic gain is selection based on individual performance within families, or combined selection. If the breeding value of the individual being considered for selection is denoted G, the mean breeding value of the population is $\acute{G}$ and the mean breeding value of the family to which the individual belongs is $\acute{G}_{fam}$, the following relation exists:

$$G - \acute{G} = (G - \acute{G}_{fam}) + (\acute{G}_{fam} - \acute{G}) = h^2_w\,(P - \text{þ}_{fam}) + h^2_{fam}\,(\text{þ}_{fam} - \mathcal{P}) \tag{4}$$

where heritability factors $h^2_w$ and $h^2_{fam}$ are coefficients of the regression of the additive genetic value on phenotypic value, and $P$ , $\text{þ}_{fam}$ and $\mathcal{P}$ represent phenotypic values of individual, family means and population mean, respectively.

The relation between $h^2_{fam}$ , $h^2_w$ and $h^2$ (the heritability of the trait) are derived by Hazel et al. (1943), and cited by Gall (1990) as:

$$h^2_{fam} = h^2 \times (1 + (n-1)r) / (1 + (n-1)t) \tag{5}$$

$$h^2_w = h^2 \times (1-r) / (1-t) \tag{6}$$

where r is the relation between breeding family members (r = 1/2 for full sibs, r = 1/4 for half sibs) t is the intraclass correlation, and n is family size.

Gall (1990) recommended combined selection as the breeding plan of choice for aquaculture production. For all practical uses this method has advantages over both individual and family selection. Expected genetic gain will be higher, and in contrast to individual selection, there will be no risk of inadvertent mating of relatives since the pedigrees of the brood fish are known (although in a closed population some inbreeding is inevitable). Recently, Gall et al. (1993) outlined different methods for estimating genetic changes from selection.

### *Inter- and Intraspecific Hybridization*

Hybridization or crosses of pure bred lines, natural strains or species utilizes non-additive genetic variation, i.e., variation caused by genes that show dominance (interaction between alleles) or epistatic effects (interaction between genes at different loci). Offspring performance may exceed the mean performance of both parents. Such effects are most often seen when crossing between groups assumed to have very different genotypes, resulting in offspring with a high heterozygosity. Productive traits and survival, and other adaptive traits may be affected. Such effects are often called heterosis, nicking effects or hybrid vigour. Heterosis is stochastic in nature and cannot be predicted, but has to be determined by performing the actual crosses and comparing the hybrid offspring to pure-bred offspring. Possible outbreeding depression was reported by Gharrett and Smoker (1991) who crossed even- and odd-year pink salmon for release. The $F_1$ hybrids returned to the hatchery in almost the same proportion as the control, but the $F_2$ hybrids showed a very low return rate as well as increased bilateral asymmetry indicating that coadapted allele complexes are disrupted by outbreeding.

Salmonid species may easily be crossed, and such crosses are done both for fisheries enhancement and for intensive production. There are two main reasons for making such crosses: (1) to obtain a higher growth rate than that of the pure species; and (2) to have a sterile fish that does not invest in gonadal growth, or that may be used for stocking rivers and lakes without risk of reproduction leading to overcrowding the environment or interbreeding with the wild fish.

Experiments and practical experience with hybridization of salmonids were reviewed by Chevassus (1983) and by Nævdal and Dalpadado (1986). The general conclusion from the literature is that species hybrids are of little value in aquaculture. They are not necessarily sterile and show only slight heterotic effects. In addition they are not usually accepted by the market because of their unknown and often peculiar appearance.

Intraspecific hybridization has also not come into common use in aquaculture, although Kincaid (1976) reported reasonably high genetic improvement by crossing pure lines (produced by several generations of sib crossings). However, the cost of keeping inbred lines, the reduced survival due to inbreeding itself, and the more predictable and greater effect of selection programs have caused this experimental series to be terminated. Crosses of natural strains with the aim of utilizing heterosis have only shown very limited success. It may thus be concluded that intraspecific hybridization is a possible means of genetic improvement, but it is probably inferior to selection.

*Chromosome Manipulation and Sex Reversal.* The main practical aim of working with chromosome engineering and sex reversal in salmonid aquaculture is to control reproduction, either by making a sterile fish or obtaining all female populations to reduce the incidence of early maturation, which is a phenomenon more common with males. The most appropriate methods are described and discussed by Thorgaard and Allen (1987) and only a brief overview will be given here (*see* Chapter 17 for more details).

*1) Polyploidy.* Polyploidy may be obtained by physical or chemical treatment in the early stages of the fertilized egg. Tetraploids may be obtained by stopping the first cell division at the moment when the chromosomes are divided. The cell will then get a double number of chromosomes, and this will persist in all cells as development proceeds. Tetraploids, however, are not very viable, and the main reason for use of tetraploid fish is to obtain a broodstock which, by back-crossing with normal diploids, gives triploid offspring. Triploids are expected to be sterile and may possibly show increased growth rate. However, triploids are more easily produced directly by heat shock during the meiotic cell divisions (which in fish take place simultaneously with fertilization). A heat shock (for example 28°C for 20 min starting 0.5 h after fertilization) will disturb the meiotic cell division in such a way that the second polar body remains within the egg cell nucleus. Thus, the resulting offspring will have two sets of maternal chromosomes and the normal single set from the male parent. Triploid salmonids perform more or less like diploids in growth rate and development. Female triploids do not develop gonads and are thus sterile. However, male triploids will, to some degree, become sexually mature, and also develop secondary sexual characteristics. Thus production of triploid sterile fish is only partly successful (Thorgaard and Allen 1987). Guo et al. (1990) found few benefits of triploids prior to sexual maturation concerning growth rate, but the growth was significant affected by maternal strain effects.

*2) Gynogenesis.* Gynogenesis is a process that may be used in two different ways, to produce pure lines or to produce all female populations. Gynogenetic offspring are produced by using irradiated sperm (UV or X-ray treatment) in which the genetic material is destroyed, but which is still able to initiate egg cleavage. Thus a haploid offspring could be produced, but haploid salmonids will die before the eyed egg stage. By interrupting the second meiotic division by physical treatment in such a way that both chromatids are restored within the nucleus, diploid individuals are produced. Such diploids have received their genetic material from the female parents, and theoretically they are homozygous at all loci (purely inbred by definition). However, crossing over between non-sister chromatids during the cell division, gynogenetic individuals are not completely homozygous. These individuals are more inbred than those obtained by several generations of sib matings and thus may be used for intraspecific hybridization. Gynogenetic fish are necessarily all females, and thus the problem of early maturing males is avoided by this technique. Triploid gynogens are sterile.

*3) Androgenesis.* Androgenesis is a process in which the genetic material of the eggs is irradiated. Thus, the offspring get all their genetic material from the male parent. To avoid non-viable haploid offspring, the first cell division is interrupted to double the genetic content of the nucleus, i.e., develop diploid individuals. Androgenesis is a possible way of redeveloping natural populations from gene banks comprised of deep-frozen sperm, but this method is still at an experimental stage of development.

*4) Sex reversal.* The sex of fish may be influenced by oral administration of hormones. By feeding female or male steroids, a population of all one sex may be obtained. However, feeding food fish with hormones may be questionable for ethical and market reasons. Thus, methods are developed where only the broodstock are fed hormones. Usually young fry are fed male hormones for a few weeks. This will result in an all male population that is composed of normal males (XY chromosomes) and functional males that genetically are females (XX chromosomes). By using such males for fertilizing eggs from normal females, all the offspring will be normal females.

It is unclear to what degree methods of chromosome engineering have been taken into practical usage in fish farming. The combination of sex reversal and triploidization seems most promising, and probably a considerable portion of the rainbow trout and possibly also salmon produced in intensive aquaculture are manipulated to control sex or maturation. It should be pointed out, however, that such treatments have no influence on the quality of the product.

*Gene Technology.* Application of gene technology for genetic improvement presupposes identification and isolation of specific genes involved in productive traits for use in broodstock evaluation programs. Such studies are still to be regarded as basic, but with a clear aim of commercial application. Isolated genes may be used in two principally different ways: (1) for production of recombinant bioactive compounds or (2) for microinjections into the newly fertilized eggs for inclusion into the genome of the recipient species. The latter process results in "transgenic" fish.

Most attention has been given to isolation of genes controlling the production of hormones. Use of genes from conspecific individuals are preferred, either to provide genetic variation or to include more copies of the same gene into the genome of one individual. Isolated genes from other species are also used for development of techniques and for purely scientific purposes. However, use of such genes in commercial production might have marketing and ethical consequences. The same type of problems will readily emerge if recombinant growth hormones are produced for inclusion in feed, especially if foreign (e.g., mammalian) genes are used.

Isolation of genes controlling disease resistance or related to stress tolerance presents promising possibilities for future aquaculture. However, until now the results of such studies have been rather inconclusive and not yet clear enough for practical application. The same conclusion may be drawn on efforts to control maturation by isolation of prolactin genes and low temperature tolerance by isolation of glycoprotein genes.

An overview of research projects started in different countries to produce gene-modified salmonids are given in the report of the ICES Working Group on Genetics (Anon. 1991). At the moment, it is impossible to foresee the practical application of gene transfer and development of transgenic fish. The large size of salmonid eggs facilitates the microinjections, but the large amount of yolk mass makes precise injection into the nucleus difficult. Early results suggest that it is not difficult to produce recipients with changed performance due to injected foreign genes, but it is difficult to have the donor's genes included into the genome of the recipient, and thus to transfer them to future generations (*see* Chapter 17).

### *Case Histories of Fish-breeding Programs*

At the World Symposium sponsored and supported by the European Inland Fishery Advisory Commission of FAO (EIFAC) and the International Council for the Exploration of the Sea (ICES) in Bordeaux, France, in 1986, several case histories of fish-breeding programs were reviewed. These programs are described in the proceedings from the Symposium (Tiews 1987): the main points are given here.

*Common Carp.* Carps have a much longer history as cultured fish than salmonids. Examples of carp (*Cyprinus carpio*) breeding programs are therefore useful in evaluation of the future of salmonid breeding. In Israel breeding programs on carps have been carried out for several decades (Wohlfarth et al. 1987), and both European and Chinese carp races have been tested. Although differences in growth rate between families indicate substantial heritability, mass selection for growth has been ineffective. Heterosis for growth rate and disease resistance has been demonstrated, and in aquaculture practice, crosses between two parental strains are used.

In the former USSR the first work on carp selection was carried out in the 1920s, and highly productive breeds have since been produced (Kirpichnikov 1981). The initial broodstocks were established by crossing local and foreign strains. Mainly, mass selection was performed, but selection for relatives and linear breeding were also carried out. However, high selection intensity caused undesirable correlated responses, for instance, abnormalities of gonads and increases in the number of spinal and cranial defects.

Presently the intensity of selection is lower, and no less than 5% of the total number of fish reared are maintained for breeding. In recent years new genetic methods have been used to aid the selection program. These include the use of genetic markers for distinguishing different strains and hybrids, diploid gynogenesis, hormonal sex transformation, and use of chemical and radiation mutagenesis to increase the genetic variation and to induce polyploidy, particularly production of triploids and tetraploids. A number of new highly adaptive and productive breeds of common carps have been developed.

*Atlantic Salmon.* A large-scale breeding program with Atlantic salmon was established in Norway in 1986 (Gjerde 1993; Gunnes 1987; Refstie 1990). This program (*see* Figure 4) is overseen by the fish breeders associations, and it is based on research on quantitative genetics carried out at research institutions since 1971. At two breeding stations, Kyrksæterøra and Sunndalsøra, 200-300 families are tested each year for the following traits: growth rate, age at maturation, food conversion, disease resistance and flesh quality, all reflecting the breeding goals of producing a fish better suited for the market and with lower production costs. These traits are combined into selection indices, and selection is based on within family performance; a selection intensity of about five percent is practiced and inbreeding is avoided. The families are kept isolated until the fish are about 15 g, then marked by freeze-branding and transferred to common ponds and later to net pens in the sea.

Selected material (smolts or eggs) are sold to multiplying stations, usually one in each county. Here the material is reared, mass selected for high growth rate, and the resulting eggs are sold to smolt producers. Selected material may also be sold directly from the breeding stations to the smolt producers. Fish in this system are subjected to very extensive fish health control. No live material is transferred from multiplying stations or other fish farms to the breeding stations. However, data on performance of the selected material are regularly collected at the multiplying stations and specific test stations to guide the effect of the selection. The program is governed by a committee with representatives from research institutions and from the aquaculture industry. The activity is supposed to be economically self-sustaining; an annual improvement rate of a 3-4% in growth rate is obtained.

The Canadian Salmon Genetics Research Program began in 1974 at St. Andrews, New Brunswick. An overview of the program was given by Bailey (1987), and a number of publications concerning genetics, physiology and salmon ranching and husbandry have been produced in addition to the results of the core project (reported in the annual reports). Results included the genetic improvement of salmon originally for sea ranching and, in later years, also for intensive fish farming. Although the return rate of sea-ranched salmon still is low, the genetic improvement obtained in productive traits for intensive fish farming after only one generation is encouraging (Friars et al. 1990; Anon. 1991).

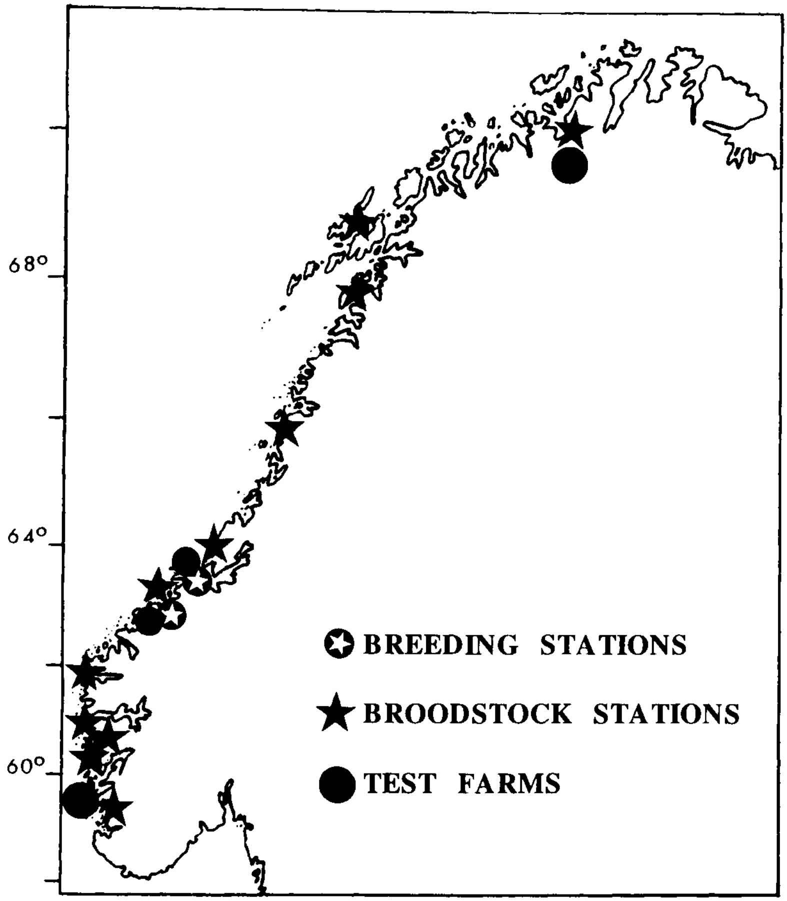

FIGURE 4. Atlantic salmon breeding system in Norway. Tagged family groups are tested for commercially important traits at selected fish farms (●) along the Norwegian coast. Based on the results obtained, family selection are carried out at the two breeding stations (✪), Kyrksæterøra and Sunndalsøra. Offspring from the selected, improved stock are transferred to broodstock stations (★) which raise the fish for broodstock and large-scale egg production. The high quality eggs are then sold to the smolt-producers and the salmon-farming industry.

*Other Species.* A 10-year selection and breeding program with coho salmon, conducted by the University of Washington's School of Fisheries in the U.S. in cooperation with Domsea Farms Inc., has produced two pedigreed lines with more than 60% improvement in weight. Family selection has been performed. Even after this selection, heritability estimates indicate that a long-term selection program could produce a further large improvement in performance (Hershberger et al. 1990).

A strain registry for trout species has been developed (Kincaid 1981, 1987) although it is not directly related to genetic improvement. In this registry a total of 293 broodstock of rainbow trout (143), brook trout (43), brown trout (42), cutthroat trout (39) and lake trout (26) have been identified and their inherent performance in culture listed. Such registries are of great help in management of inland fisheries and for development of intensive and extensive aquaculture.

## GENETIC MANAGEMENT OF WILD SALMONID STOCKS

The stock concept has been used for a long time in salmonid management. Earlier, the stock structure within a species was based on biological information as discussed in Chapter 3. At present, many salmonid species have been analysed for genetic population structure, at least in some regions. The information clearly demonstrates the existence of subgroups or stocks which are more or less genetically distinct.

The rational management principle, from a genetic point of view, is to treat each stock separately according to the important life history characteristics that are necessary for a healthy and sustainable population. The maintenance of the genetic characteristics of the stock usually require a minimum number of spawners contributing to the next generation and good nursery habitat for the egg and juvenile survival before smolts or fry migrate to seawater. Any human activities that influence the different stages of salmonid life history potentially cause some genetic changes in the stock in question.

Genetic changes in the characteristics of wild salmonid stocks are usually considered to be harmful. Genetic diversity at both population and species levels is of greatest importance for future evolution and adaptation to a changing environment. The present genetic structures of populations are results of long-time periods of genetic differentiation, and the conservation of genetic variation among populations and species is therefore one of the first challenges with respect to utilization and preservation of the salmonid resources. Conservation of genetic resources in a long term perspective does not mean "freezing" of the present genetic structure, but maintaining (or increasing) the present level of genetic variation resulting in high evolutionary potential.

### *Effects of Harvest Management on Wild Stock Genetics*

The harvest of salmonid resources has always been controversial, and a matter of struggle for the harvest between different groups and interests. With the exception of the historical native harvest, the commercial fisheries of Pacific salmonids, in the sea started about 170 years ago at the west coast of the American continent. The rapid development of fisheries for export caused large pressure on and interest in utilization of the resource. The Atlantic salmon, on the other hand, has a rather long history of salmon harvest controversies. In contrast to the public ownership of the rivers in North America, the salmon-sustaining rivers in many European countries have been and are still privately owned. In many cases there is a long history of controversy connected to the interest of salmon harvest in the rivers compared to saltwater fisheries. For example, the first written court records in Norway regarding a salmon dispute occurred in the 1300s. In this case, the farmers having fishing rights in the upper part of the River Suldalslågen repeatedly fought to stop the organized salmon fishery in the river mouth initially owned by the church. This controversy continued almost for 600 years and this situation is almost typical for many important salmon harvest issues.

In principle the anadromous salmonids can be harvested at different stages of their life histories. Historically, the harvests were carried out in connection with spawning and during the spawning migration in the rivers and in the estuaries. With development of larger fishing vessels, new technology in fish location and effective fishing gear, the importance of coastal and high sea fisheries increased enormously. At present, the salmonid resources are the bases for commercial fisheries both in international areas and in water bodies under national jurisdiction. This has created a very high harvesting pressure, and rational management requires international cooperation and agreements on harvest quotas. The value of salmon resources and the subsequent high fishing pressure on every possible harvest segment will continue to be controversial and represent one of the most important challenges to proper management and conservation of the genetic resources within salmonid species (Bentsen 1991).

From a genetic point of view, the subdivision of the different salmonid species into a large number of stocks with their own gene pools, represents a difficult management problem. The intraspecific genetic variation (as described earlier) is possibly a result of evolution and adaptations to various environmental conditions over a long time. The stocks therefore constitute specific genetic resources which are irreplaceable and must be conserved. The amount of genetic variation at the population and the individual level is also the raw material for further evolution and adaptation to a changing environment. The maintenance of this variation can mean the difference between healthy stocks in the future or their extinction.

The persistence of a specific salmonid stock is dependent of several factors. The most important ones are, however, the existence of freshwater spawning areas and a sufficiently high number of spawners contributing to the next generation. For wild salmonid reproduction, unpolluted, undisturbed nursery areas for juveniles are necessary conditions. The actual spawners contributing to the next generation must represent the genetic characteristics of the population in question and the number of spawners must be high enough to avoid problems related to inbreeding. If these requirements are met, the excess fish could, in theory, be harvested at any stage and area of salmon distribution.

Potentially many kinds of human activity, especially river regulation, water pollution and fishing pressure, can cause the extinction of a wild salmonid stock or an unfavourable genetic change. With reference to the comprehensive treatment of biological characteristics of Pacific salmon stocks and species by Ricker (1972) and Taylor (1991), it is obvious that single-stock management represents an enormous task. Many of the important life history characteristics (time of run, age at maturity, sea migration pattern) of the stocks are at least partially determined by genetic factors and thus are sensitive to harvest strategies. This is clearly documented in Pacific salmonids where catch statistics on different species and in different areas are available for several decades. Ricker (1981) summarized some of the information at the STOCS Symposium in 1980. Mean weight in the commercial catches were compared in the period from 1951 to 1972. In all species, including pink, chum, sockeye, coho and chinook salmon, a decline in mean weight was observed in most of the statistical catch regions. The largest decreases (20-40%) were detected for chinook, coho and pink salmon. The corresponding data in the same period for chum and sockeye salmon was in the range 5-10% decrease. For all species there were changes in the average age at return. The changes observed were explained by the fishing pressure in the growing areas and especially the gear used. There was no correlation with temperature or salinity during the same period and the changes were most likely due to a selective fishery, which favoured catches of larger fish having higher prices in the market. Data on selective fishing for Atlantic salmon is not as complete as for the Pacific salmon, but the principle is, however, the same and several observations exist support similar phenomena.

As the high sea fisheries in the ongrowing areas and coastal salmon fisheries during spawning migration are harvesting a mixture of many stocks, management based on the single-stock principle is very difficult or almost impossible. There will always be a risk of overharvesting of weak stocks, which could then become extinct. For this reason, the harvesting of different stocks is more rational if carried out when the stocks have segregated out according to their estuaries or rivers late in the spawning migration. For this reason, safe stock identification methods are urgently needed for management of the fisheries.

Recent methods used to characterize and identify stocks (Allendorf and Utter 1979; Ferguson 1980) demonstrate the complex stock structure of species populations. When clear genetic differentiation was detected, these methods could also be actively used in management of the salmonid resources. Such work was initiated in the early 1980s by Utter and colleagues aiming on developing an analytical approach to identify different stocks in a mixed stock fishery (*see* Pella and Milner 1987 and references therein). The methods are based on multi-locus information (achieved by electrophoresis) for the wild stocks in the area. Genetic baseline data showing genetic differentiation between stocks are required in the genetic stock identification (GSI) method. Mixed samples from an offshore fishery are analysed by electrophoresis and compared to the baseline stocks using maximum likelihood statistical techniques. The computed programs provide estimates of the contributions of the different stocks in the mixed sample in question, which than can be used to regulate the fishery.

The first GSI programs were carried out on chinook salmon on coastal areas of the U.S. (Milner et al. 1985) and on pink salmon in British Columbia (Beacham et al. 1985). These programs clearly demonstrated the potential for large-scale application of GSI methods to the other Pacific salmonids (Utter and Ryman 1993). At present, a large scale GSI program has been established covering the area from California to Alaska incorporating studies on chinook, chum, pink and sockeye salmon. Five different governmental agencies in the U.S. and Canada are involved, and the electrophoretic analyses are carried out at six different laboratories. The different components of this large scale GSI approach for Pacific salmon have been discussed recently by Shaklee and Phelps (1990). They emphasized the requirements on all the different stages of the analyses including genetic baseline information of reference stocks, sample collections, interpretation of electrophoresis variation and the details of the mixed stock analysed, and computer output in terms of stock contribution in a mixed sample (Shaklee et al. 1991). The development of nomenclature and standardization of the methods between different areas and laboratories is very important for an optimal application and reliable use of this method in management.

The level of genetic variation (at least as detected by electrophoresis) in Atlantic salmon seems to be much lower compared to Pacific salmon. A large number of samples from both sides of the North Atlantic has been analysed (*see* Ståhl 1987; Davidson et al. 1989) but to date there have been no attempts to use the GSI approach in Atlantic salmon management.

### *Hatchery Programs and Enhancement of Wild Salmonid Stocks*

Artificial reproduction of salmonids has been understood for a long time (*see* Chapter 1). Early hatchery activities were usually motivated by the desire to increase distribution area by stocking fry into water bodies not available for natural immigration. Later there were numerous stock transfers between rivers and areas (even continents) in an attempt to increase the run of salmonids in a specific river or establish a salmonid population of fish having a larger size. Such stock transfers were mainly performed before the recent development of knowledge of the genetic structure of salmonid population, and the genetic effects of the mixing of stocks over large distances are not known. With reference to the discussion above, it is likely that this activity has had a significant impact on the present population structure at least in some areas and for some species.

Transfers of Pacific salmon into Atlantic waters have been tried in several occasions. In his review, Harache (1992) concluded that survival rates of Pacific species are generally lower than in the species' original range, and that such transfers have not contributed considerably to any fishery and have had little or no influence on native stocks or species.

The new information about the genetic variation and stock structure of wild salmonids suggested new management requirements and conservation approaches (Gharrett and Smoker 1993*a*). The main task is now to achieve maximum harvest of the natural populations and, at the same time, conserve the genetic resources they represent for the future. In that context, the goal of the hatchery program must be properly defined and the genetic design and requirements must be carefully chosen. Clearly, as discussed below, the genetic strategies are different in many cases and the potential effects on wild salmon stock genetics will vary.

Human activities such as selective harvest, salmon habitat destruction and pollution, have been shown to have genetic effects on natural populations. Hatchery practices also have the potential of influencing the genetics of wild salmon. Allendorf and Ryman (1987) discuss in detail the principles of genetic effects of hatchery activities. When the fish produced are released into natural waters, it is of great importance to reduce the effects of hatcheries. The use of locally adapted stocks for broodstock, the effective number of wild parental fish used, and maintenance and control of the genetic variation and characteristics of the hatchery stock, are all specific aspects that should be controlled and carefully evaluated.

As noted, the hatchery-production goal must be linked to a genetic strategy. Whenever hatchery fish are released into wild populations, there will be genetic interactions between hatchery and wild fish gene pools. The outcome of this interaction will depend on the status of both hatchery and wild fish and the environment. For this reason, different kinds of hatchery activities and releases are discussed separately.

*Self-sustaining Wild Stocks.* This category includes endemic salmonid stocks which have been affected by human activities only to a minor degree, but which have lost some of their ability to reproduce naturally. The spawning areas are not destroyed by pollution or water regulation, and the number of spawners is large enough for natural reproduction and preservation of the genetic characteristics and the level of genetic variation within the stock. These populations possibly represent the most diverse and valuable salmonid stocks. They are often adapted to a specific environment and have specific life history patterns.

In such cases, the population should be maintained and protected naturally by reducing all potentially negative human impacts. All hatchery activities should be avoided, and the harvest strategies must include preservation of subgroups and spawners in such a way that fishery selection is minimized.

*Enhancement of Wild Stocks.* Traditionally, hatcheries have been build to compensate for loss of natural reproduction mainly due to construction of dams or pollution in specific river systems. This has occurred almost everywhere in the world and in most countries large hatchery programs have been initiated to compensate for reduced natural reproduction and to support various salmonid fisheries. In some cases, there is some natural reproduction in the river, but this is considered to be too small from an economical point of view, and the production of the population is enhanced by artificial methods. Riddell (1993) provided a comprehensive overview of reasons for conserving natural populations, means of conservation and principles for management to conserve genetic diversity.

In cases where there are available wild salmonid spawners, it is of fundamental importance that the enhancement is based on the locally adapted stock. This stock is assumed to be the most productive under the local conditions, and this approach can minimize unwanted genetic changes in the wild stock from the point of view of conservation.

As discussed earlier, all hatchery practices are likely to induce some genetic changes. These include intended as well as unintended changes, and have been well documented (*see* references in Skaala et al. 1990; Hindar et al. 1991; Utter et al. 1993). Several examples are also presented in the review by Allendorf and Ryman (1987) who discussed various important genetic principles in connection with hatchery production of fish for release. With the preservation of the wild local stock in mind, it is important to use spawners that reflect the genetic characteristics of the wild population. Based on the genetic variation detected within the stock by enzyme electrophoresis and other genetically determined traits, this approach normally requires a minimum number of broodstock for each generation. The critical number of spawners used [*see* Allendorf and Ryman (1987) for detailed discussion] is associated with the *effective* number of parental fish, which is also dependent of the ratio of males and females. An optimal ratio between males and females is assumed to be 1:1 and a minimum number of 50 spawners in founding a hatchery stock. Under these conditions, the inbreeding coefficient is less than 1% per generation. A much larger number of spawners, minimum 100 males and 100 females is recommended (Allendorf and Ryman 1987). In cases where the main goal is to preserve long-term genetic variation, several hundreds of spawners are needed for each generation (*see also* Chapter 4).

Preservation of the genetics of a wild stock while at the same time enhancing the total production of the stock by hatchery releases, seems to be quite difficult to achieve (*see* Ryman 1991). The requirements of a large number of wild spawners in each generation, genetic control to minimize unwanted genetic changes (adaptations to hatchery conditions) during artificial production, and the need of a selective fishery for hatchery fish returning to the release sites are enormous tasks that are difficult to accomplish. Co-existence of a wild reproducing stock and a genetic hatchery-based strain (even based on founders from of the same stock) is, in practical terms, very unlikely within the same river system. The experiences so far, especially when considering small stocks with few available wild spawners, are that genetic changes are occurring in the hatcheries and that these changes are gradually being incorporated into the wild spawning stock (Skaala et al. 1990; Crozier 1993). A synthesis of the literature on genetic and ecological relations of supplementing natural stocks with hatchery-reared salmonids has been prepared by Steward and Bjornn (1990), and additional examples and case studies may be found in their report.

The only way to reduce this process is to mark all the hatchery fish and perform a selective fishery to prevent them from migrating into the spawning sites and contributing to the next wild generation. Spawners for artificial propagation must each year be selected from the "true" wild spawning population according to the basic principles discussed above. In most cases, this genetic strategy will be impossible to carry out in practice, and enhancement by hatchery activities will cause genetic changes in the wild population with the risk of destroying the genetics of this population. A new hatchery-based stock is gradually developed that will replace the native wild stock, which if a locally adapted stock, will be irreversibly lost.

*Endangered Stocks.* In some cases salmonid environments have been changed so rapidly and seriously from habitat destruction and pollution that the population is not able to reproduce naturally. In other cases fishing pressure has reduced the spawning population to very low numbers and inbreeding depression threatens the existence of the stock.

With regard to stocks with inbreeding problems, every effort should be directed to maximize the number of spawners and the conditions for juvenile survival in the nursery areas. If the stock is likely to become extinct due to inbreeding, transfer of spawners from other wild stocks should be considered. Artificial crosses using milt from wild males from the endangered stock and females from another stock in the area, will possibly represent an increased "natural straying" and gene flow of great importance. Offspring of this kind could be produced in the hatchery and released as juveniles or smolts in the river to supplement the reduced wild juvenile generation.

The supplemental approach is not possible in cases where the reproduction and nursery habitat have been destroyed permanently or for a short period. Many pollution situations, such as acid rain, have wiped out populations almost completely. Reduction of the pollution is, however, possible in the future, resulting in improvements and recoveries in riverine environments. For this reason, it is important to actively use the hatchery to maintain the original population, as far as can be done, before it vanishes.

In cases where there can be no natural reproduction, at least for a few years, it may be possible to preserve, through hatchery production, the essence of the wild stock even though this will entail significant genetic changes brought about by the selection regime of the hatchery. The hatchery must, of course, be populated by the original stock.

A local fishery may then be continued and some of the fish will probably attempt to reproduce even under unfavourable environmental circumstances. If environmental conditions improve, the recovery of the natural reproduction of the stock may be possible. In such cases, it is very important to keep a hatchery stock representing the genetic profile of the original local stock for several generations and simultaneously perform annual releases in the wild. If such living gene banks are not possible, one should carry out a milt collection program and keep the material cryopreserved for later use (Gausen 1993; Wildt et al. 1993).

*Rehabilitation of Stocks.* In many river systems pollution has been intense for decades and there may have been no salmonid reproduction for a very long time. Such examples are found in the high population density and industrial areas of central Europe and other industrial nations. The earlier stocks of Atlantic salmon adapted to those rivers (such as the Seine and Rhine) have become extinct, and it is not possible to re-establish stocks with the original genetic characteristics.

Extensive dam construction for production of inexpensive electric power has also caused salmon stocks to become extinct. The Atlantic salmon run in the Connecticut River was possibly one of the largest in North America about two centuries ago. During the Industrial Revolution almost every tributary in the water system was used for production of energy, and by about 1920 all salmon had disappeared from the river (Jones 1988). Pacific salmon stocks in the Columbia River have suffered from similar hydro-electric project development during this century.

A more recent problem is the effect of acid rain on salmonid reproduction. In many areas both in Europe (especially Southern Norway) and eastern North America, freshwater and anadromous salmonid populations have disappeared due to acidification. This process has been occurring over the past few decades and several other stocks are endangered.

In all such cases rehabilitation of salmonid stocks is of great importance. Improvement of the river environment is likely to take place and will create opportunity for restoration of naturally reproducing salmonid stocks. No broodstock from the native or original stock is, however, available as founders for the restoration. Rehabilitation of the former stocks is therefore not possible and a genetic strategy to establish a new salmonid stock must be considered.

The genetic components of rehabilitation programs have been discussed in detail in one of the STOCS Symposium synthesis papers (Krueger et al. 1981). Those authors recommended two different strategies with respect to choice of broodstock founders. One approach is to collect wild spawners from a large number of genetically different stocks and carry out many crosses to maximize genetic variation. The offspring are then released into the environment in question, natural selection allowed to work, and the most fit genotypes should survive at the highest rate. The new adaptation process is dependent on the amount of genetic variability available and will probably take considerable time.

The second strategy from Krueger et al. (1981) involves use of preadapted genetic material. Existing salmonid stocks from natural refugia and environments similar to the rehabilitation area, are likely to contain some of the genotypes needed. Such populations are often found near the rehabilitation site. Use of broodstock from these local stocks actually increases natural gene flow from remaining wild stocks, and may increase the probability of a successful restoration of a new salmonid population.

The rehabilitation of a salmonid fishery by hatchery activity in an area where salmonid populations have been destroyed is much simpler than establishing a new wild, self-sustaining stock. Considering the large number of salmonid transplantations throughout the world, there have been very few examples of a successful establishment of new natural reproducing stocks. This is possibly because the salmonid populations are locally adapted through many generations. The problems involved and the cost and effort needed are illustrated by the restoration program of Atlantic salmon in the Connecticut River (Jones 1988). The major initial problem of the program was that the original gene pool of the Connecticut salmon was lost. Initially, offspring from many stocks from other areas were used in releases, but very few fish have returned to the river. The program has been going on for 25 years, and the limited results so far underline the difficulties involved and the costs needed to perform a successful rehabilitation program. For similar examples see Krueger and May (1991).

*Salmon Ranching.* In this type of hatchery activity the main goal is simply to produce large numbers fish for release as a basis for a fishery in a geographically determined area or for harvest when the mature fish return to the release site (*see* Chapter 14). This may be carried out as a public activity, by private enterprise or by fisher's cooperatives. In this case the broodstock that is most economically valuable could be chosen and the genetic concerns encountered on conservation of wild gene pools will be of less importance.

Ranching of salmonids is often classified as a form of aquaculture, and the term extensive aquaculture often applied. The genetic principles connected to ranching are more parallel to intensive culture or farming of fish and are treated in more detail in the next section.

In his review of salmon ranching, Isaksson (1988) summarized the principles of ranching and discussed ongoing ranching activities for many salmonid species in different parts of the world. Especially in the north Pacific, the numbers of salmonids released annually is enormous. The pink and chum fry releases in Japan and Russia constitute the greatest numbers (in billions), but also coho, sockeye and chinook salmon (in millions) are produced in hatcheries and released as smolts by Canada and the U.S. The releases are based on a large number of public hatcheries, private ranching companies and fishermen's cooperatives, and are mainly aimed at supporting a specific fishery in a given region of the sea (*see* Isaksson 1988 for details and references).

The activity of Atlantic salmon ranching is quite limited compared to the magnitude and importance of Pacific salmon releases. Traditionally, hatcheries have usually been based on specific river stocks for enhancement and compensatory release programs. The activities in Europe have been based to a large degree on private ownership of the rivers and more linked to anglers' organizations and sport fisheries interests. The largest releases of Atlantic salmon smolts during the last decade have been in the Baltic Sea, mainly to compensate for destroyed natural smolt production in Sweden and Finland. These releases have been very successful for the sea salmon fishery in the Baltic proper, but have had a detrimental effect on the remaining wild Baltic stocks (Ackefors et al. 1991).

Atlantic salmon ranching in Iceland has been developed from the work carried out at the Kσllafjordur Experimental Fish Farm. The activities started in the 1960s and at present a number of private ranching enterprises have been established (Isaksson 1988). The ban on salmon fisheries in the sea offers the possibility of a more profitable harvest of the returning salmon compared to other nations where the sea fishery accounts for the largest part of the salmon catch. The development of salmon ranching as a private industry in Iceland also suggests new research opportunities to optimize ranching operations, and it is no surprise that research aimed at selection programs for specific ranched stocks of Atlantic salmon have been initiated in this country.

### *Selection Programs and Salmonid Ranching*

*Principles.* As mentioned in previous sections, many of the life history characters of salmonids have a genetic component (Jonasson 1993). Heritability estimates for economically important traits are basic information required for breeding plans for farmed fish. The principles of genetic selection are of course valid for all traits that are under genetic control or influence and these methods can be used in a breeding programs for salmon ranching stocks. Many important components must be considered and evaluated in developing a commercial breeding program (*see* Shultz 1986; Gjedrem 1993).

For private ranching operations, genetically based improvements that minimize the hatchery costs, increase seawater survival and growth, and increase the return rate of the ranched fish are of great importance. In cases where a hatchery is operating in a watercourse or at a release site with no wild salmon stock, the use of a well performing ranching stock could be of vital importance for the industry. The well developed animal breeding methods should be used in connection with commercial ranching operations and such projects should be designed to minimize potential genetic impacts on valuable wild stocks.

*Review of Case Histories.* Most of the quantitative genetic research on salmonids has focused on identifying important traits as bases for breeding programs. There are very few, if any, cases where a full scale commercial breeding program of ranched salmonids has actually been carried out.

The early works of Donaldson (Donaldson and Menasveta 1961 in Donaldson 1968) demonstrated that several traits connected to survival and productivity must be under genetic control. They observed improved performance characters like growth rate and precise homing migrations.

Similarly, Carlin (1965) found that the total yield of different families used in a stocking program with Atlantic salmon differed considerably. These findings were confirmed later (Ryman 1970) indicating that differences in performance traits between families were reflected in differences in recapture frequencies, recapture weight and mortality frequencies, where genetic factors contributed significantly to these variations.

The genetic research program at the NASRC started in 1974 and was originally founded to develop improved strains for Atlantic salmon ranching. Although the original aim has been very difficult to accomplish due to the low general recapture frequencies, the program has achieved significant improvements in the return frequencies in offspring generations compared to the founder broodstock.

In recent years there has been new interest in developing breeding programs in Atlantic salmon. Gjedrem (1986) presented a research approach for estimating heretabilities for important traits (such as growth rate, maturation and recapture frequencies) as important elements of a breeding plan. Based on estimates obtained on farmed salmon, it seems obvious that a large potential for improvement exists. A program based on this design, including family testing and selection, has been initiated in Iceland as a part of a Nordic research program (Jonasson 1994; Isaksson 1994). A large number of individuals has been tagged and are identified to family after return to the release sites (Figure 5). Unfortunately, the return rates have been quite low during the last years due to unfavourable seawater conditions. The between-family variation in the return rate is very large and the possibilities are very good for performing a breeding program to yield rapid improvements in the stocks or in returns (Isaksson 1989; Jonasson 1994).

*Effect on Wild Stocks.* For wild salmonids, natural selection acts on all life history stages. Spawning competition and survival of the offspring in the freshwater stages are probably fundamental elements of stock adaptations which are removed in the hatchery situation. Any hatchery operation will probably include other selection forces different from those of the wild, and may cause some new adaptations or genetic changes.

In the hatchery where there is a breeding program for ranching, the freshwater natural selection factors have been removed. The hatchery conditions will possibly

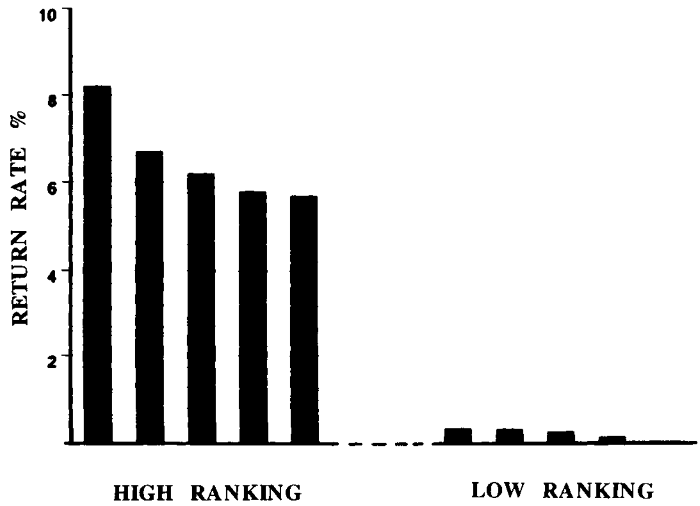

FIGURE 5. Atlantic salmon ranching and variation in return rate. In the Icelandic large-scale ranching experiment, 141 different families were tagged and released. The mean return rate was 2.2% and ranged from 0 to 8.2% for the families tested. The diagram shows the return rate for the five highest and the five lowest ranking families (one family with no returns, far right). (Redrawn and modified from Jonasson 1994).

represent other selection factors which will change the genetics compared to the wild fish. The broodstock are, however, selected from the returning fish having the best saltwater performance (growth rate, return rate). The overall performance of a wild salmon stock is a compromise or balance between selection forces in both salt and fresh water. For a ranched stock, the saltwater selection (neglecting hatchery selection) is of largest importance and this stock could therefore, at least in theory, develop an advantage in the feeding areas compared to wild fish. Large releases of fish from a superior ranching stock could cause some negative impacts on the wild fish in the feeding areas if the food availability is limited (Hayashizaki and Hitoshi 1991).

The selected ranched stock will, after some generations, differ genetically from the wild stocks. Straying of ranched spawners to wild, locally adapted stocks increases the possibility for gene introgression and breakdown of adapted wild gene complexes. Natural gene flow between genetically different wild stocks is believed to have a biologically important function, but a large number of ranched strayers and an increase in gene flow imply a high risk of changing the genetic characteristics of the wild populations. At present, natural straying estimates vary from species to species, and are usually in the range between one to 10%. Most straying estimates are based on recaptures of tagged fish, which do not mean that the straying fish are actually caught at the spawning site during spawning. Thus the effective strayers in genetic terms, are the individuals that contribute their genes to the next generation. This number is much lower than the observed number of overall strayers. The estimates of reproductive success from natural wild strayers and ranched strayers are urgently needed for evaluation of this potential problem.

The straying frequencies of ranched salmon are dependent on the release strategies, location of release site, and especially the efficiency of recapture of returning fish at the release site. With optimal release strategies and imprinting of smolts, the returning fish seem to home precisely to the site of release, even without use of fresh water. In commercial salmon ranching, efficient harvesting is a crucial matter for the economical results of the enterprise. As many as possible of the returning fish are to be caught at the release site, so that the number of ranched fish straying to wild salmon rivers remains small.

Large releases of hatchery fish compared to the population size of natural stocks also indirectly affects the wild stocks. This is mainly due to an increase in fishing pressure in the feeding areas. The problem of stock-specific management of a mixed stock fishery has been discussed above, and in such cases the poor stocks are most sensitive to over-exploitation. The risks of unwanted effects are of course dependent on the fishery regulations; control of catches and quotas could be set to protect the wild stocks.

The large compensatory smolt release and fishery in the Baltic Sea is an example in which the remaining wild stocks are endangered. Only few naturally reproducing wild salmon stocks are left in the Baltic, and about 80% of the total salmon smolts are presently of hatchery-reared origin (Ackefors et al. 1991). The development of a salmon sea fishery in the Baltic proper accounts for 85% of the total catch, and decreases in wild salmon production have been observed.

The present knowledge of salmonid life histories and the importance of imprinting and precise homing indicates that the potential genetic conflict between wild populations and ranched stocks can be avoided. Private ranching, including selection programs for well performing strains, should be located in areas without important wild stocks. The broodstock used for founding a ranched stock should be collected from the nearest wild stocks in order to reduce potential genetic effects due to straying. The smolt production and release methods should maximize site imprinting. Efficient catch methods should be used for returning fish in order to minimize straying to wild salmonid populations.

In many parts of the world, private salmon ranching is actually carried out at sites located far from important wild salmonid stocks. In the Icelandic ranching industry, most of the ranching farms are located in coastal areas a rather short distance from the feeding grounds in the high seas. Very little straying to wild salmonid stocks has been detected

(Isaksson 1988, 1989). New approaches to protect the wild Atlantic salmon stocks left in the Baltic Sea (Ackefors et al. 1991) point to the possibilities of maintaining a sea harvest, but changing the areas of fishing. A ban is proposed on the international fisheries in the Baltic proper, which could be replaced by a coastal fishery in defined areas where large numbers of smolts have been released by the "delayed release technique". This method has at least doubled the survival and recapture frequencies, and straying to wild salmon rivers has been shown to be very limited (Eriksson and Eriksson 1991). By locating the release sites in coastal areas far from natural salmonid stocks (Skilbrei et al. 1994) and carrying out the main harvest when the stocks are separated, the problems connected to mixed stock fisheries are possibly reduced. This approach suggests separate monitoring and conservation programs for each wild stock, and the sea fisheries to be concentrated on hatchery fish in defined coastal areas to reduce the straying frequencies.

This "coastal salmon ranching" is possibly a rational approach in cases where it is important to develop a conservation strategy for wild salmonid stocks and at the same time maintain a salmon harvest or salmon ranching in coastal areas. Even with the very low straying frequencies so far observed, it is, however, important to estimate the extent of straying in all new experiments in other areas and under different environmental conditions. The final evaluation of new ranching approaches must be based on straying frequencies of ranched fish and estimates of interbreeding with wild stocks in nearby rivers.

### *Interaction Between Farmed Fish and Wild Stocks*

Many animal species were domesticated thousands of years ago, and this includes some fish species. Salmonids as domesticated animals are, however, more recent. All farmed animals are of wild origin, and have gradually adapted to the farm environment over a long time. They are thus very different from their wild relatives. These genetic changes are further increased by selection programs that emphasize economically important traits so that farmed strains often are unable to survive and reproduce in a natural environment.

Several research studies have been and are being carried out on farmed salmonids and the heritabilities of economically important traits (*see* above). However, only one large scale commercial breeding program has been initiated so far. This program, carried out in Norway by the Salmon Farmers Association, has been in operation for only four generations of selection (based on characters discussed above). The salmon farming industry is producing large amounts of Atlantic salmon, and during the production period there are numerous technical accidents allowing large numbers of farmed salmon to escape. These are found in substantial numbers in the sea fisheries and even ascending with wild salmon in rivers. This situation has created a public and scientific controversy about the potential risk of genetic impacts and the need for conservation of wild salmonid gene pools. The specific case chosen for discussion is Atlantic salmon, but the principles and genetic aspects are of course, in general, valid for other salmonids as well as other farmed fish species.

*Genetic Differentiation.* All salmonid species are, under natural conditions, divided into many more or less genetically distinct populations with their own gene pools and life histories. The degree of genetic differentiation varies from species to species, and the genetic diversity is believed to be of immense biological importance for further survival and new adaptation to changing environmental conditions. Preservation of the genetic variability within the different species is one of the most important challenges for future management (Nelson and Soulè 1987; Utter et al. 1993).

All farmed salmonid stocks have been founded initially by collecting eggs from wild spawners. The actual number of founders and the number of genetically different stocks they were taken from define the genetic variability and the genetic range available to the farmers and breeders (Shultz 1986). With respect to Atlantic salmon, the breeding program in Norway was based on family and river stock testing, including 40 different stocks. Based on growth rate and farm performance, the best ranking strains and families were selected and constituted the raw material for constructing the farmed salmon strain in Norway. The goal of the breeding was, of course, to improve the important traits by selectively changing the genetics of the strain.

The present farmed Atlantic salmon strains are different from the original wild stocks in several important characters. A recent pedigree analyses through the selected generations (Gjedrem et al. 1991) demonstrated that only a few originally wild river populations are actually contributing genetically in the present farmed strain. This is possibly due to an effective selection process causing genetic changes in relatively short time. In many respects the development of a farmed salmon stock is an adaptational process to an artificial environment without natural reproduction. To what extent domestication and selection have reduced or removed the ability of the farmed salmon to reproduce under wild conditions is unknown.

Such information is needed for an proper evaluation of the problem. The breeding for a high performance salmon strain has been carried out for very few generations compared to other farm animals which have lost their ability to live and reproduce under natural conditions. Although, at this early stage of domestication, the farmed salmon have probably reduced ability to compete and reproduce in the wild, they may still do so. The possibilities of crossings between farmed and wild spawners, most likely between farmed females and wild males (including small sneakers), will cause an increase of foreign genetic material into local salmon populations.

*Possible Effects on the Wild Gene Pools.* Escapement of large numbers of farmed salmonids may effect the natural gene pools in several ways (Gudjonsson 1991; Lund et al. 1991; Webb et al. 1993). Mature salmon of farmed origin are caught in many rivers during the spawning run. They are, however, more abundant in the lower part of the rivers and are poorly able to manage waterfalls. Farmed Atlantic salmon ascend the rivers much later than the wild fish, and in some cases there is a risk of redigging wild spawning redds, which clearly will reduce wild salmon reproduction (Lura and Scegrov 1991). Specific adaptations are often associated with timing of spawning, with hatching, and survival conditions for the larvae and juveniles. Late spawnings of farmed salmon are likely to be unsuccessful, but with a harmful effect on wild reproduction. This is mainly an ecological interaction and could cause some genetic effect (inbreeding depression) if only a few wild spawners are responsible for the new wild generation (Thomas and Mathiesen 1993).

The potential genetic effects of farmed salmonids spawning under natural conditions are dependent of many factors. The first requirement for a genetic effect is that the escaped farmed fish can reproduce under natural conditions and interbreed with the wild fish. Reliable estimates of reproductive success of farmed salmon, as contributing genes (farmed offspring, hybrids between wild and farmed spawners) in the next generation are, at present, not available. The lack of reliable information on reproductive success and introgression from farmed salmonids are possible reasons for the scientific controversy in this field (Bentsen 1991; Cross 1991; Egidius et al. 1991; Hindar et al. 1991; Mork 1991; Heggberget et al. 1993).

In theory, there are several possible effects depending on the genetic status of the wild population (extent of adaptation, number of spawners, size of wild spawners, cultivation activity) and the genetic differentiation of the farmed strain. The number of escaped Atlantic salmon spawners in the rivers in Norway varies from zero to 60-70%, usually late in the spawning season (Lund et al. 1991). For this discussion, we assume that the escaped farmed salmon occur at a frequency of about 10-20% at the spawning sites, and have a normal spawning behaviour and reproductive ability.

For a genetically adapted stock, the invasion of spawners of farmed origin represents a threat to genetic integrity. In cases where there have been no releases of hatchery fish and the watercourse is unaffected by pollution and water regulation, the population constitutes an important and valuable genetic resource. It has been shown that farmed spawners are inferior to the wild fish with respect to spawning success. The farmed male fish seem to be less active compared to wild males (measured as fighting ability and number of completed spawnings) in spawning tank experiments (Cross et al. 1991); the spawning success of farmed females was, in this study, about half of that of the wild females. In a more realistic situation, on the wild spawning grounds, the fish can move around and find suboptimal spawning sites and partners where the spawning competition is not as intense. The spawning competition pressure is also dependent on the number and size of wild spawners, so that the spawning incidence of farmed salmon will be suppressed in a population with many wild spawners of large size. Successful spawning is, however, likely to take place between farmed females and wild males with farmed males ranking low in the competition hierarchy.

In the case of a successful spawning of farmed salmon, the depth of the redds and sites could be critical for development of the eggs and hatching at the optimal time for suitable temperature and availability of food for new fry. Thus optimal timing of such processes are linked to the specific environmental conditions that obviously vary among localities. The wild fish are adapted to such environmental conditions and the offspring from strayers or farmed fish are therefore strongly selected against at the different life history stages. The selection forces are also very strong among the alevins and juveniles. Only a small fraction of the newly hatched yolksac larvae survive to the smolt stage and migrate to the sea. It is reasonable to assume that offspring of farmed origin are more sensitive to this selection and that the frequency in the juvenile population will decrease with age.

Hybrids between farmed and wild fish could have a better performance for some traits compared to the parental fish. Such heterosis (or hybrid vigour) effects are sometimes observed in the first generation, but disappear in the next generations. Under these conditions, nonadaptive genetic material would be introduced into the locally adapted population and the genetic characteristics of the population changed. The introgressed genes could have a negative overall effect on the population and reduce performance. If the introgression rate of foreign genes is high, this process would likely to cause a breakdown of the adaptive gene complex with serious consequences for the wild population in question. The result is another salmon stock, genetically different and nonadaptive under those environmental conditions, which actually threatens the future existence of the population.

In many rivers, it is questionable that the present salmonid stocks really represent the original stock in genetic equilibrium, adapted to the specific environmental conditions. There is a long history of stock introductions and transplants for almost all salmonid species in different parts of the world. At the same time, the salmon habitat has been changed due to human pollution and water regulation, often followed by massive releases of hatchery fish of mixed stock origin. Only during the last decade, have there been new requirements with respect to the type of broodstock that should be used in various rivers. In many cases there have been observed changes in general traits and in the performance of the stock, but no reliable genetic information exists. It is therefore reasonable to believe that such stocks do not represent unique, native gene pools, but are populations in a more genetically unstable situation. This suggests that although the strong selection forces connected to spawning and survival of offspring, discussed above, are working in this case, the results of gene introgression from farmed salmon are even more unpredictable.

As discussed earlier, spawning areas have been destroyed in many rivers, and the salmon fisheries are fully dependent of hatchery releases. Only 5-10 years ago, there were many examples of river owners and angler clubs that every year bought excess smolts from the salmon farming industry and released them in natural reproducing Atlantic salmon rivers. These fish clearly have a greater chance of passing their genes onto the next generations compared to a limited number of escaped spawners from the farming industry. The degree of genetic influence that new gene flow from farmed individuals will cause in the "wild" population is impossible to predict. In cases where the number of natural spawners are very low (inbreeding problems) such gene introgression of farmed genes can even be beneficial for the population.

## CONCLUSIONS

When reviewing the literature, the main impression is that the knowledge of the genetics of salmonids and its application for breeding schemes is considerable. However, for practical management of wild populations and for proper use of salmonids in culture, some areas must be emphasized as especially important for future research; some of those areas are as follows:

Additive genetic factors play an important role for several productive traits such as growth rate, age at maturation, carcass traits, and flesh pigmentation, for example. However, little is known about the complex interaction between genetics and environment for such traits. Control of age at maturation appears to be very complicated and specific experiments must be designed to reveal the causes of variation in this trait.

It is of crucial importance to know whether individual or family differences in growth rates are caused by different intake of feed or different feed utilization rates. The fact that the cost of feed is the highest expense in intensive culture emphasizes the importance of research on this question.

The effects of non-additive genetic factors are not well understood. This understanding has implications for use of intraspecies hybridization, which may be an alternative breeding method. Non-additive genetic factors are also related to the effects of inbreeding, which are somewhat unknown.

Considerable effort has been made on chromosome engineering and on sex reversal both for studying the principles of genetic control, and for use in salmonid farming. The practical application of some of these techniques has been demonstrated while others are still being studied (*see* Chapter 17). Further research is needed for safe and effective utilization of these techniques.

A great deal is known about the population structure of salmonids in nature and the genetic variation within and between population units. Much less is known about isolation mechanisms as well as effects of new or changing environments. These items are also of great importance with regards to the effects of escaped farmed salmon on natural gene pools as well as those of sea ranching and stock enhancement (Windsor and Hutchinson 1990). Will gene pools be restored when influences by introgression by foreign genes are terminated? What mechanisms are involved, and do adaptations to local environmental conditions exist? To answer such questions large-scale field experiments are needed. One way to study the phenomena is to use morphological and biochemical genetic markers (Seeb et al. 1990; Utter and Seeb 1990). Single-locus DNA analyses offer new tools for such studies in addition to those already developed from the field of enzyme studies.

The study of covariation between a single gene in a genotype, e.g., enzyme genotypes, and productive traits represents an interesting, although difficult task for future research. Several indications of the existence of such covariation have been reported, but until now quantification has been difficult, and utilization in breeding programs has not been practised. Genetic variation in disease resistance has received considerable interest in recent years. Such resistance is now partly used in breeding programs, and will probably be an important part of any program aimed at improving strains for all types of culture.

The largest challenge in the future is to integrate the different fields in salmonid genetics in order to develop a complete strategy for each species with respect to conservation of the genetic resources and their use in aquaculture. At present, several scientific controversies exist which are based on specific interests in salmonid genetics. Application of genetic principles in wild salmonid management, enhancement of stocks, and salmonid ranching are fundamental in importance for preserving genetic variation under future changing environmental conditions. The success of salmon farming and the possibilities for improvement of farmed strains are dependent on conservation of the genetic resources in wild stocks.

Several areas of genetic information are lacking that are needed to develop a sustainable strategy for management of salmonid genetic resources. The most important information is possibly the extent and significance of interactions between wild, genetically distinct populations and hatchery or farmed populations. Detailed knowledge of isolating mechanisms and the genetic dynamics between such populations are urgently needed for future management of salmonid stocks.

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## Chapter 12

# TRANSPORTATION AND HANDLING

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## INTRODUCTION: THE TRANSPORTATION OF LIVE FISH

### *Historical Perspective*

The subject of fish transportation is as old as aquaculture itself, dating back to the first records of fish culture as practiced in ancient China and the Roman Empire. Little is known of the actual methods and equipment used to transport fish in those times but presumably fairly short distances were involved. Even so, a great deal of ingenuity and resourcefulness must have been needed to compensate for the lack of today's mechanized hauling equipment and technical information on fish physiology (McCraren 1978).

The first records of fish transportation over long distances and on a wide scale date from more recent times – 1874 and the pioneering work of Dr. Livingston Stone of the U.S. Fish Commission, the forerunner of the U.S. Fish and Wildlife Service. In that year, Stone transported 35,000 shad (*Alosa sapidissima*) fry in open milk cans by railway about 3,000 miles from the Atlantic coast of the U.S. to the Sacramento River on the Pacific coast (Stone 1874). The general fishery management problem faced by the Fish Commission still exists today, that is the hatcheries rearing the fish were relatively few in number and often widely separated from the waters to be stocked. Stone and others devised hand-operated aerators, filters to remove slime, and showed that adding ice to the water increased its oxygen carrying capacity and reduced the oxygen consumption of the transported fish. By 1879, Stone had transported striped bass (*Morone saxatilis*) fry from New Jersey to California in a railroad baggage car using some of these newly developed techniques. The young fish were held in milk cans cooled with ice and Stone hired fellow passengers to help with the hand-operated aerators. During later trips in

1882, a new aerating device was used that was considered a great improvement — a pan with small holes drilled in the bottom. The pan was repeatedly pushed down into the water and lifted up, resulting in a cascade of aerated water into the fish tank (Shebley 1927).

By the early 1880s, the basic biological principles of successful fish transportation had become widely recognized: sufficient aeration to re-oxygenate the water, a relatively constant temperature, water kept free of slime and excretory products, and the necessity to protect the fish from injury as "fish with broken fins and scales did not keep well" (McCraren 1978). Fish culturists were also well aware of the fact that only fish in good initial condition should be selected for transport, and that the water would not become as fouled with excrement if the fish were not fed for several days before hauling. The practice of adding ice to the hauling tank water was also widespread because of the earlier practical experience showing its dramatic effect on oxygen consumption.

Aside from the methods used by Dr. Stone, a few records exist of other early techniques used for transporting fish over long distances. Some of these were quite innovative. Moore (1887) reported that he had successfully shipped live sole from England to America in swinging glass aquaria suspended in the cargo hold of a freighter. The swaying motion of the ship helped aerate the water. Holder (1908) reported that lining milk cans with sponges effectively protected transported fish from injury. He also fitted the caps with a perforated false bottom to help aerate the water as it splashed around — a portent of the Fearnow pail.

By 1910, reliable motor-driven pumps had become available and were successfully used for aeration in shipping black bass from California to the Philippine Islands by steamship. By the 1930s, equipment such as Fearnow pails, ice, and hand aerators was rapidly being replaced by tanks with motorized water circulation systems, refrigeration, and compressed air for aeration. In 1935, smallmouth bass (*Micropterus dolomieu*) were shipped from Maryland to South Africa, first by freighter and then by airplane, using this type of mechanized equipment (Harrison 1938). However, eggs and fry were also still being hauled by truck using only the passive aeration provided by Fearnow pails (McCraren 1978).

The principal fish hauling method that had come into use during this time, however, was the railroad car, custom built and reinforced to aerate and carry large amounts of water. This was the so called "fish car" era — a period of roughly 50 years from about 1890 to 1940. During this time, trains literally crisscrossed the U.S. transporting enormous numbers of freshwater and anadromous fish and shellfish to the waters to be stocked. Records show that the species hauled included pike, perch, shad, whitefish, carp, catfish, trout, salmon, lobster, and dungeness and blue crab (Leonard 1979). At the peak of the fish car era, the trains were sometimes kept busy hauling fish day and night. In 1930 alone, the cars and their biologist attendants travelled more than

500,000 miles. During the ensuing years as highways and motor vehicle reliability were improved, the forerunners of today's modern hauling trucks were developed and the railroad fish cars gradually fell into disuse. In 1947 the last remaining fish car was taken out of service and an era came to an end (Leonard 1979).

### *Importance Today*

Since about 1960, a literal information explosion of technical data on fish physiology and the effects of water quality conditions has occurred. This advanced knowledge, together with the reliable mechanized equipment now available, has enabled transportation methods to improve to the point that high survival rates can now almost be taken for granted, even when fish are transported long distances. As a result, fish hauling and distribution operations have become an indispensable part of aquaculture and fisheries management. In commercial aquaculture, for example, smolts reared in freshwater hatcheries on shore are routinely transported by helicopter, airplane, or boat to remote seawater net-pen sites for grow-out. In recreational fisheries management, fry, fingerlings, or yearling fish are routinely trucked hundreds of miles to remote lakes and streams for stocking or even planted by air drop from light aircraft or helicopters. Eyed eggs are now routinely shipped around the world to serve fisheries management needs in other countries, as are live tropical fish for the aquarium industry. A particularly impressive example of modern day fish transportation is the massive Columbia River smolt-hauling operation conducted each year by the U.S. Army Corps of Engineers. Twenty million or more migrating juvenile coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*), and sockeye (*O. nerka*) salmon, and steelhead trout (*O. mykiss*) are collected in the upper Columbia and Snake rivers, barged or trucked hundreds of miles around the dams, and then released into the lower river to continue their seaward migration. Depending on the species, smolt survival can approach 100% (Ceballos et al. 1991).

The physiological concepts that serve as the basis of the methods being used to achieve the present high level of fish survival during transportation are also applicable to other aspects of intensive fish culture. These include moving fish on site, sampling ponds or raceways to determine growth and feed conversion rates, and handling fish during inventories (counting). The most important of these physiological concepts and methods will be discussed in the following sections of this chapter.

## PHYSIOLOGICAL CONCEPTS IMPORTANT TO FISH TRANSPORTATION

In practice, success in transporting live fish comes down to solving the physiological problems caused by the economic necessity to haul relatively large numbers of fish in a relatively small volume of water. A life-support system must be provided that will prevent the adverse water quality changes that would otherwise quickly occur, and meet the physiological requirements of the transported fish. A working knowledge of basic aspects of fish physiology is fundamental to the rational design of the required system.

### *Respiration and Oxygen Consumption*

An understanding of the physiology of respiration and oxygen consumption, the critically important processes by which fish exchange and transport the dissolved oxygen (DO) and carbon dioxide ($CO_2$) in the hauling tank water, is basic to designing a life-support system that will allow consistently high survival rates to be achieved. Because the DO concentration in water is normally limited to a maximum of about 12–13 mg/l (compared with the approximately 300 mg/l available to air breathing animals), fish must move a relatively large volume of water over their gills to obtain enough oxygen to support life. Salmonids accomplish this by mouth and opercular movements when at rest and by ram ventilation (open mouth) when swimming at moderate velocities (Smith 1982). Gill anatomy is arranged such that the flow of water over the gills is opposite to the flow of blood through the gills, making it theoretically possible for up to 80% of the DO to diffuse into the blood. The amount of oxygen diffusing through the gills is also controlled to some extent by hormonal mechanisms. When fish are stressed (e.g., by handling), epinephrine (adrenaline) is produced which alters blood circulation through the gills to increase the effective surface area and thus the amount of gas that can be exchanged. Unfortunately, this physiological response also increases the osmotic influx of water, which then must be excreted. Blood electrolytes are unavoidably lost in the resulting diuresis and the resulting ionoregulatory dysfunction is part of the reason for the delayed mortality commonly referred to in fish-transport operations as hauling loss.

After oxygen diffuses through the gills into the blood, it is bound by haemoglobin (Hb) molecules in the erythrocytes and circulated into the capillary beds of the tissues. The Hb-$O_2$ bond is weakened by the high concentration of $CO_2$ present there (Bohr effect), and the oxygen is released to diffuse into the cells where its concentration is low. The process by which the $CO_2$ produced in the tissues is carried in the venous blood back to the gills and eliminated into the water also has implications important to success in fish transportation. Unlike oxygen, $CO_2$ is readily soluble in blood plasma, haemoglobin is not needed for its transport, and the diffusion of $CO_2$ from the plasma through the gills into the ambient water is also rapid. Thus, the normal blood $CO_2$ concentration in salmonids and other active coldwater fish is quite low and the Bohr effect is correspondingly large (begins at low blood $CO_2$ levels). In fish-transport operations, the dissolved $CO_2$ in the hauling tank water should not be allowed to increase above 20–30 mg/l to prevent blood $CO_2$ concentrations from rising (hypercapnia) and the resulting Bohr effect from impairing adequate oxygen transport to the tissues. The oxygen-carrying capacity of fish Hb will also decrease if the blood pH declines (Root effect). In hypercapnia, the Root effect will compromise oxygen transport to the tissues only if the

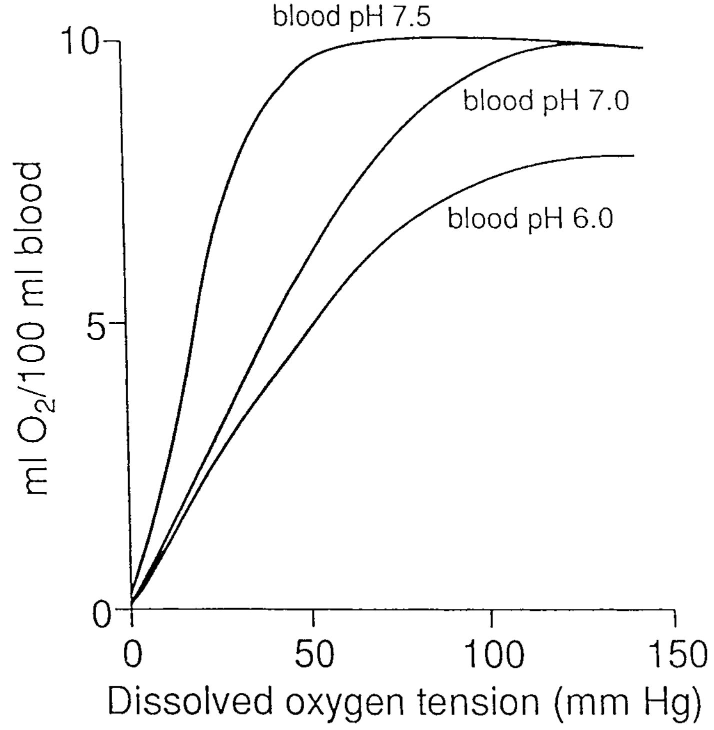

FIGURE 1. If blood pH decreases (acidosis), the oxygen tension required to saturate salmonid haemoglobin increases substantially. Blood pH 7.5, normal conditions. Curves for pH 7.0 and 6.0 illustrate the progressive reduction in $O_2$ carrying capacity due to acidosis if blood $CO_2$ or lactic acid levels rise during transport (redrawn from Wedemeyer et al. 1976).

blood $CO_2$ increase is large enough to cause metabolic acidosis. Increased blood lactic acid concentrations (hyperlacticemia) because of excitement and excessive swimming activity can more easily overwhelm the blood buffering capacity causing acidosis and reduced oxygen transport to the tissues (Figure 1). Respiratory stress from the Bohr and Root effects can be minimized by fish-handling protocols that will prevent hyperlacticemia (by minimizing excitement and swimming activity), and by an aeration system designed to strip out dissolved $CO_2$ as well as provide an adequate supply of dissolved oxygen. In practice, these are two of the most important considerations in meeting the physiological needs of transported fish.

The oxygen consumption of salmonid fish in intensive culture is affected by a number of biological and environmental conditions and can vary from 100 to 600 $mg \cdot kg^{-1} \cdot h^{-1}$ or more (Liao 1971). Two of the most important of the conditions affecting the oxygen consumption of transported fish are excitement and stress. Haskell (1941) was probably the first to document the rapid drop in DO that normally occurs during the first half-hour after loading and correctly attributed it to the effect of excitement caused by handling. Barton and Schreck (1987) showed that the oxygen consumption of physically stressed juvenile steelhead trout immediately more than doubles and that it remains high for at least another hour. In fish transport operations, this initial oxygen demand should be anticipated by starting the aeration system 5 to 10 min before fish are loaded to ensure that the hauling tank water is saturated.

Water temperature and swimming activity level can also strongly affect oxygen consumption during hauling. The actual magnitude of these effects was first documented by Brett (1973) for juvenile sockeye salmon. The baseline curve (resting activity level) of Figure 2 shows the effect of water temperature alone on oxygen consumption. As illustrated, increased swimming activity increases oxygen consumption more than warmer water temperatures. Oxygen consumption rates calculated for juvenile salmonids at swimming activity levels typical of hatchery raceway conditions are tabulated in Table 1 for a range of water temperatures. Note, however, that aeration systems must be designed to supply sufficient DO to support the oxygen consumption of transported fish over a wide range of swimming activities as well. If the DO in the hauling tank water is not replaced rapidly enough and begins to decline, salmonids respond physiologically by moving water over their gills at a faster rate. Unfortunately, this also reduces the proportion of the DO that can be extracted during each pass from the theoretical 80% maximum to as little as 15% (Smith 1982). As a result, the fish expends more and more energy to get less and less oxygen. At a DO of about 1 mg/l, the bioenergetic cost of obtaining and utilizing the oxygen becomes greater than the energy derived from its consumption and tissue anoxia, unconsciousness, and eventually death will result.

Fortunately, many of the biological and environmental factors that act to increase oxygen consumption can also be manipulated to lower it during transportation. Of the methods currently employed, reducing the water temperature (hypothermia), fasting the fish before hauling, and using anaesthetics to reduce excitement and swimming activity are the most common. These will be discussed in some detail in a later section of this chapter.

### *Toxic Excretory Products*

When fish are transported, their excretory products immediately begin to accumulate in the distribution tank. Relatively large amounts of ammonia and carbon dioxide together with smaller amounts of urea, creatine, creatinine, uric acid and other nitrogenous wastes are continuously produced (Smith 1978). Of the nitrogenous waste excreted by salmonid fish in fresh water, about 80% is ammonia. The remainder is usually ignored because it is so much less toxic than ammonia. Salmonids fed typical dry diets produce 25-35 g of ammonia per kg of feed consumed (Westers 1984). Thus,

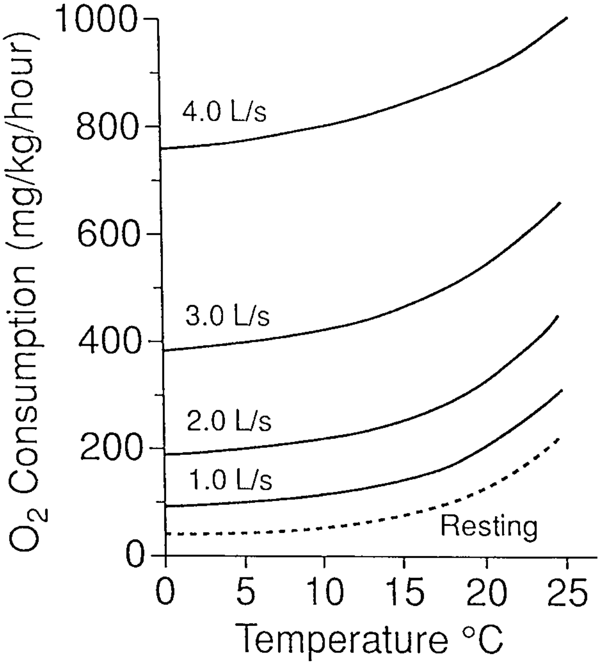

FIGURE 2. Effects of swimming activity (body lengths per second; l/s) and water temperature on the oxygen consumption of juvenile sockeye salmon (redrawn from Brett 1973).

depending on time since last feeding and the length of the trip, it is not uncommon for the total ammonia concentration in a fish distribution tank to reach 10 mg/l or more (Smith 1978).

After ammonia is excreted, the hydrogen ions present in the hauling tank water convert it into a mixture of the ammonium ion ($NH_4^+$), which is essentially nontoxic to fish, and un-ionized $NH_3$ which is toxic. The concentration of $NH_3$ that the fish will be exposed to is determined by the pH and, to a lesser extent, the water temperature and total dissolved solids (TDS) concentration. Chemical analysis measures the sum of both forms of ammonia present (i.e., $NH_3$ and $NH_4^+$), and the amount of toxic $NH_3$ actually present must therefore be calculated. Selected values for the percentage of $NH_3$ that will be present at the pH and water temperatures of most interest in salmonid smolt hauling operations, calculated from the equilibrium equation for $NH_3/NH_4^+$, are tabulated in Table 2. For transport operations in hard water areas, a correction for TDS may need to be applied (Messer et al. 1984). For example, if chemical analysis shows that the total ammonia concentration is 10 mg/l, the water temperature is 10°C and the pH is 7.0, the amount of un-ionized (toxic) $NH_3$ present would be only about 0.02 mg/l; well within the

**TABLE 1**

Oxygen consumption ($mg \cdot kg^{-1} \cdot h^{-1}$) as a function of water temperature of juvenile coho salmon and chinook salmon at typical hatchery raceway swimming activity levels[a].

| **Fish size (grams)** | **Oxygen consumption at temperature of (°C):** | | | |
|---|---|---|---|---|
| | **5** | **10** | **15** | **20** |
| 0.1 | 222.8 | 420.4 | 594.3 | 803.0 |
| 0.5 | 163.0 | 307.6 | 434.9 | 587.7 |
| 1.0 | 142.5 | 268.9 | 380.2 | 513.7 |
| 1.5 | 131.7 | 248.6 | 351.4 | 474.8 |
| 2.0 | 124.6 | 235.1 | 332.4 | 449.1 |
| 3.0 | 115.2 | 217.3 | 307.2 | 415.1 |
| 4.0 | 108.9 | 205.5 | 291.5 | 392.6 |
| 5.0 | 104.3 | 196.8 | 278.2 | 375.9 |
| 10.0 | 91.2 | 172.1 | 243.2 | 328.6 |
| 25.0 | 76.3 | 144.0 | 203.6 | 275.1 |
| 50.0 | 66.7 | 125.9 | 178.0 | 240.5 |
| 75.0 | 61.7 | 116.4 | 164.5 | 222.3 |
| 100.0 | 58.3 | 110.1 | 155.6 | 210.2 |

[a] calculated from $O_2 = KT^nW^m$ (Liao 1971)

4-h maximum safe exposure level of 0.1 mg/l presently recommended for salmonids (Environmental Protection Agency 1986). If the pH was 8 (at the same water temperature), the concentration of $NH_3$ formed would increase to 0.18 mg/l. Fortunately, the $CO_2$ that is also continuously being produced by the fish will usually maintain the pH below 7, especially in the waters of low total hardness typical of smolt hauling operations in North America. Thus, the concentration of toxic $NH_3$ in a hauling tank will normally remain below the accepted 4-h acute exposure limit of 0.1 mg/l even if the total ammonia concentration reaches 10 mg/l or more as in the above example.

As mentioned, the second toxic excretory product that can accumulate in fish distribution tanks is carbon dioxide. In general, salmonids produce about 1.4 mg of $CO_2$ for each mg of oxygen that they consume (Colt and Tchobanoglous 1981). Thus, the amount of $CO_2$ produced ($mg \cdot kg^{-1} \cdot h^{-1}$) by salmonids consuming only average amounts of

TABLE 2

Percent of total ammonia that will be in the toxic $NH_3$ form over the range of pH and water temperature of interest in smolt hauling operations[a].

| pH | Water temperature (°C) | | | |
|---|---|---|---|---|
| | 5 | 10 | 15 | 20 |
| 6.0 | 0.01 | 0.02 | 0.03 | 0.04 |
| 6.2 | 0.02 | 0.03 | 0.04 | 0.06 |
| 6.4 | 0.03 | 0.05 | 0.07 | 0.10 |
| 6.6 | 0.05 | 0.07 | 0.11 | 0.16 |
| 6.8 | 0.08 | 0.12 | 0.17 | 0.25 |
| 7.0 | 0.13 | 0.18 | 0.27 | 0.40 |
| 7.2 | 0.20 | 0.29 | 0.43 | 0.63 |
| 7.4 | 0.32 | 0.47 | 0.69 | 1.0 |
| 7.6 | 0.50 | 0.74 | 01.08 | 1.60 |
| 7.8 | 0.79 | 1.16 | 1.71 | 2.45 |
| 8.0 | 1.24 | 1.83 | 2.68 | 3.83 |
| 8.2 | 1.96 | 2.87 | 4.18 | 5.93 |
| 8.4 | 3.07 | 4.47 | 6.47 | 9.09 |
| 8.6 | 4.78 | 6.90 | 9.88 | 13.68 |
| 8.8 | 7.36 | 10.51 | 14.80 | 20.07 |
| 9.0 | 11.18 | 15.70 | 21.59 | 28.47 |

[a] calculated from % $NH_3$ = 100/(1 + antilog[pKa - pH]), TDS correction not applied. In most fish transport situations $CO_2$ will accumulate too rapidly for physiological compensation to occur so millimolar quantities of sodium bicarbonate or sodium sulphate are sometimes added to the hauling tank water to offset the decrease in blood-buffering capacity and help maintain the blood pH (Haswell et al. 1982).

oxygen can be quite large (cf Table 1), and $CO_2$ could reasonably be predicted to accumulate rather quickly in hauling tank water. In practice, concentrations of 20-30 mg/l within 30 min after loading are not unusual unless preventive steps are taken. Chronic exposure to concentrations greater than 15-20 mg/l is considered undesirable because blood pH disturbances (acidosis) and respiratory stress from the Bohr and Root effects begin to occur at higher levels (Klontz 1993). Experience has shown that fish under hatchery raceway conditions can tolerate moderately elevated dissolved $CO_2$ concentrations because the rate of increase is usually slow and the blood-buffering system can compensate for the hypercapnia by increasing the plasma bicarbonate concentration sufficiently to maintain the blood pH and prevent acidosis.

As previously discussed, if the blood $CO_2$ concentration is allowed to rise significantly the oxygen-carrying capacity of salmonid Hb will be greatly depressed because of the Bohr and Root effects. The magnitude and relationship of these effects to actual DO and $CO_2$ concentrations in the water was first documented in salmonids by Basu (1959). He showed that the DO required to provide sufficient oxygen to the tissues to support a moderate swimming activity level increased from about 6 mg/l if little or no $CO_2$ was present to more than 11 mg/l if the dissolved $CO_2$ concentration was increased to 30 mg/l. Thus, the usual recommendation that salmonid fish in distribution units will have adequate oxygen as long as the DO does not fall below about 80% of saturation must be qualified. In practice, if dissolved $CO_2$ levels are not kept well below 30–40 mg/l, the blood oxygen carrying capacity will be depressed to the point that even high DO concentrations may be insufficient to prevent tissue hypoxia.

### *Physiological Stress*

The physiological systems of fish can be severely challenged or stressed by many of the unavoidable practices involved in intensive fish culture. These include collection, loading, and transportation, and in the case of smolts, the rapid water chemistry changes that occur when transported fish are released directly into seawater. The term "stressor" or "stress factor" is used to identify the physical, chemical, or biological challenge that requires the compensatory physiological response by the affected fish. The stress response is the sum of all the physiological changes that occur as fish react to challenges such as these and attempt to compensate (Wedemeyer and Goodyear 1984).

To survive stressful environmental challenges, physiological systems must react and adjust in various ways. If the altered physiological state can compensate for the challenge in question, acclimation will occur and the probability of survival will be increased. Thus, an understanding of the stress response, and the limits of environmental change to which fish can acclimate through this response, is important to developing the scientifically based procedural guidelines needed to identify and mitigate the adverse effects of fish-transport operations on health and physiological quality. For example, the corticosteroid hormone response that occurs has recently been used to show that the stress of the initial capture and loading operations is more severe than the hauling process itself, both in transported salmonids and in warmwater species (Maule et al. 1988; Robertson et al. 1988). This is important because the effects of individual sublethal challenges are cumulative and will eventually reduce survival even though separately they do not exceed physiological tolerance limits (Barton and Iwama 1991). Similar kinds of physiological information can be used to develop procedures to minimize the stressful effects of other aspects of intensive fish culture.

The physiological changes that occur as fish attempt to compensate for the effects of environmental alterations are similar in many ways to those in all higher animals (Wedemeyer et al. 1990). During a stressful event such as collecting, loading, and transporting, the hypothalamic portion of the brain stimulates the pituitary to release adrenocorticotrophic hormone which, in turn, stimulates the interrenal cells in the anterior kidney to produce corticosteroid hormone (cortisol). Stress also stimulates the chromaffin cells, also found in the anterior kidney, to release catecholamine hormone (epinephrine). Gill circulation is altered to increase gas exchange and a series of other secondary blood and tissue alterations, such as hyperglycemia and reduced blood-clotting time, begin to occur. These physiological changes are basically adaptive in nature and normally enable the fish to physiologically compensate and improve its probability of survival. However, if the stress is too severe or becomes chronic the altered physiological state may eventually become maladaptive and fish health will be debilitated. For example, the altered gill circulation that increases the effective surface area to facilitate gas exchange also results in an increased osmotic influx of water that must then be excreted. The blood electrolyte imbalances that occur because of the ions lost in the resulting diuresis can become life-threatening and result in delayed mortalities if prolonged. The immunosuppression occurring as a side effect of the corticosteroid hormone response can also become a source of mortality if latent infections are activated (Maule et al. 1989; Barton and Iwama 1991). In the case of transported smolts, essential performance characteristics such as migratory behaviour and ability to osmoregulate in seawater may be adversely affected as well resulting in reduced ocean survival (Maule et al. 1988; Schreck et al. 1989). Effects such as impaired migratory behaviour are probably of little practical significance in smolts transported to seawater net pens for commercial aquaculture. However, the initial corticosteroid hormone changes, which can affect disease resistance, and the ionoregulatory dysfunctions, which can be mitigated to improve survival, are of considerable practical significance in smolts transported either for aquaculture or for conservation purposes.

A number of the many blood and tissue chemistry changes that occur as fish react to the collection, loading, and transport process have been used as methods to measure the severity of hauling stress and the time needed for recovery after release. The most practical of these methods can be found in Wedemeyer et al. (1990).

## BASIC TRANSPORT AND HANDLING TECHNIQUES

### *Anaesthetics and Hypnotic Agents*

Anaesthetics and similar drugs can be helpful in reducing excitement, swimming activity, and the overall metabolic rate of transported fish, which in turn lessens physiological stress, scale loss, oxygen consumption, and $CO_2$ and ammonia production. Using such drugs, rainbow trout (*Oncorhynchus mykiss*) have been successfully transported at two to three times the normal weight of fish per unit volume of water (Piper et al. 1982). However, if drugs are to be used routinely, care must be taken to ensure that only compounds licensed for fisheries applications are selected (Meyer and Schnick 1989), and that proper dosages have been calculated. For anaesthetics and hypnotics, the proper dosage is one that produces light sedation rather than anaesthesia because it is important that essential physiological functions such as osmoregulation and gill respiratory exchange not be affected (Summerfelt and Smith 1990). Also, equilibrium must not be affected. If drug-treated fish sink to the bottom of the hauling tank, they may suffocate or be drawn against the screens, causing scale loss and preventing adequate water circulation (Wedemeyer et al. 1985).

Originally it was thought that the main benefit of anaesthetics and hypnotics was to slow metabolic rates and thus reduce oxygen consumption and carbon dioxide production. However, when such agents are used at sedative concentrations they can also mitigate physiological stress due to excitement and handling, and slow swimming activity as well. The latter is a significant help in reducing scale loss — a serious cause of mortality in smolts transferred directly into sea water (Bouck and Smith 1979). Sedation also helps prevent other injuries due to excitement, such as broken fins, that would ordinarily occur during hauling.

Of the many drugs tested as transport additives, the hypnotics: sodium seconal, sodium amytal, quinaldine, etomidate, metomidate, and the anaesthetics, 2-phenoxyethanol (2-PE) and tricaine methanesulfonate (MS-222), have received the widest use (Amend et al. 1982*b*; Dupree and Huner 1984; Pennell 1991). Sodium seconal or sodium amytal used at 8 mg/l, and quinaldine at 2 mg/l reduce the swimming activity and oxygen consumption of a variety of species during hauling, but these compounds have not been cleared for use on fish for human consumption and so are illegal except for experimental use (Summerfelt and Smith 1990). Quinaldine at 2 mg/l also does not effectively mitigate the corticosteroid stress response (Davis et al. 1982). Etomidate, the ethyl derivative of its parent compound propoxate, effectively controls adverse physiological changes in a variety of transported fishes when used at 0.1-0.2 mg/l — either with or without added 1% salt. At anaesthetic concentrations (0.4-0.5 mg/l), etomidate can cause bradycardia, EKG abnormalities, and elevated blood pressure (Fredricks et al. 1993). However, at the reduced dosage used in transportation, etomidate-treated fish suffer few physiological side effects and recovery is complete within a relatively short time after release (Amend et al. 1982*b*; Davis et al. 1982).

Metomidate (Marinil®), the methyl derivative of propoxate, was originally developed as a short acting anaesthetic for swine and is presently cleared (in Canada) for certain fisheries applications as well. The anaesthetic dose for salmonids is 4–5 mg/l. As a transport additive for smolts, metomidate is used at the tranquilizing dose of 0.1–0.2 mg/l (Pennell 1991). Metomidate has few adverse side effects and work is presently underway to license it for fisheries applications in the U.S.

The anaesthetics, 2-PE and MS-222, have both been used as transport additives for a number of years. Although 2-PE provides the additional benefit of being a mild fungicide, prolonged skin contact may be harmful to human health and so registration efforts to license it for routine use are unlikely. MS-222, originally synthesized as a local anaesthetic for humans, is now approved for fisheries applications in several countries. Juvenile rainbow trout and other non-anadromous salmonids can be successfully transported sedated with MS-222 at about 25 mg/l (Piper et al. 1982). Higher concentrations (50–100 mg/l) are not recommended because they both anaesthetize the fish and stimulate oxygen consumption. An undesirably long recovery period after release from the hauling truck is also required.

In smolt hauling operations, MS-222 added at 25 mg/l causes loss of equilibrium and is not recommended. In contrast, at 10 mg/l MS-222 provides significant physiological benefits as a transport additive. Equilibrium is not affected, and swimming activity and oxygen consumption both decrease; even at loading densities as high as 24% (0.24 kg/l, 2 lb/gal). Typical oxygen-consumption rates of spring chinook salmon smolts are 210 $mg \cdot kg^{-1} \cdot h^{-1}$ in untreated hauling tank water, and 190 $mg \cdot kg^{-1} \cdot h^{-1}$ when 10 mg/l MS-222 is added. In mixed-species hauling (spring chinook and steelhead smolts, 1:1 ratio), typical oxygen consumption rates are 210 $mg \cdot kg^{-1} \cdot h^{-1}$ for fish in untreated water, and 160 $mg \cdot kg^{-1} \cdot h^{-1}$ with added MS-222 (Wedemeyer et al. 1985).

Although anaesthetic or hypnotic drugs can make it possible to increase the fish loading density by a factor of two or three times the normal weight per unit volume of water, these agents should probably not be used solely for this purpose or to compensate for basic deficiencies in water quality (Piper et al. 1982). Drugs are legitimately used, however, to decrease the amount of excitement and scale loss, a serious problem in smolts released directly into seawater (Bouck and Smith 1979). As a precaution, a recovery period of several days should always be allowed before any drug is used a second time on transported fish and human skin contact should always be minimized.

### *Fasting*

Withholding food for a period of time prior to transportation is a well-known technique used to decrease both the rate of oxygen consumption and the amount of carbon dioxide, ammonia, and feces produced (Westers 1984). However, fishes are evolutionarily adapted to intermittent feeding and considerable time may be required for fasting to produce a noticeable physiological effect. For example, rainbow trout require about 48 h without food before their standard metabolic rate even begins to decline (Dickson and Kramer 1971). After about 60 h of fasting, oxygen consumption decreases by about 25%. Phillips and Brockway (1954) showed that at least 63 h of fasting is required to reduce the ammonia excretion of rainbow trout by 50%. For brook trout (*Salvelinus fontinalis*), about 72 h without food is required to decrease the standard oxygen consumption and continued fasting has no further practical effect (Beamish 1964). Falconer (1964) originally proposed the general rule that salmonids smaller than about 0.1 g should be starved for at least 2 days before they are transported and larger fish for at least 3 days in order to lower their metabolic rates a sufficient amount to produce a practical effect.

At present, the recommended practice is to fast all salmonids for 48–72 hours before transportation.

### *Hypothermia*

Water temperature is one of the most important of the environmental factors that can be manipulated to improve the survival of transported fish and the benefits of hypothermia have been recognized by fish culturists since at least the 1800s (McCraren 1978). As previously discussed, water temperature controls metabolic rate so that hypothermia reduces both the amount of oxygen required by fish and the amount of toxic excretory products they produce. Because the $Q_{10}$ for most physiological processes is about 2.0, reducing the water temperature by 10°C would be expected to reduce the oxygen consumption and waste production of transported fish by a factor of about two. However, Phillips and Brockway (1954) found that cooling the hauling tank water by only 8°C decreased the oxygen consumption of transported rainbow trout by about 50% while Meehan and Revet (1962) found that lowering the water temperature by 6°C reduced the oxygen consumption of transported sockeye salmon fry by only 20%. More recent work by Smith (1977) has shown that the $Q_{10}$ for the energy metabolism of salmonids actually averages somewhat lower (1.4–1.9) than the expected value of 2.0 and also that the relationship between energy metabolism and temperature is linear only over the range of 3–18°C.

In addition to reducing oxygen consumption, hypothermia also assists in mitigating aspects of the physiological stress response. Lowering the water temperature from 10°C to 5°C reduced hyperglycemia by about 30% in spring chinook salmon smolts trucked at a loading density of 12% (0.12 kg/l, 1.0 lb/gal) (Wedemeyer et al. 1985). Unfortunately, chilled water offers little protection against the blood electrolyte depletion that seems to be a major factor in the delayed mortality that can result from hauling smolts. Thus, the usefulness of hypothermia as a fish-transport control measure is probably limited to reducing oxygen consumption and ammonia production.

Practical experience has shown that, within limits, cooling hauling tank water by 1°C will allow an actual increase in the fish loading density of about 10% (Piper et al. 1982). For this reason, hauling tank water is now usually cooled somewhat prior to loading fish as a matter of routine. Refrigeration systems or the use of chlorine-free ice to lower the water temperature to 5–7°C, together with aeration starting about 5 min before loading are currently the most widely used methods of reducing metabolic rates and compensating for the initial rapid oxygen consumption that occurs due to excitement immediately after loading begins (Smith 1978). The amount of cooling needed can be judged from the difference between the hatchery water temperature and the temperature of the receiving water. However, the hauling tank water is generally not chilled more than about 10°C below ambient temperatures. Also, if the receiving water is more than 10°C colder than the hauling tank water, it is best to acclimate the fish to the new water temperature over a period of several hours. Rapid changes of less than 10°C (that are within overall temperature tolerance limits) are usually well tolerated on purely physiological grounds. However, the stress response that does occur, though mild, may still be sufficient to activate latent infections such as bacterial kidney disease if the fish have been previously exposed to pathogens (Wedemeyer and Goodyear 1984).

### *Light Intensity*

Reduced light intensity can also be useful in mitigating transport stress. Spring chinook salmon smolts subjected to a hauling challenge at twice the normal loading density of 6% (0.06 kg/l, 0.5 lb/gal) under darkened conditions showed a 25% reduction in the physiological stress response compared with smolts challenged under normal daylight (24 ft-candles). Specifically, both the hyperglycemia and hypochloremia normally caused by handling and crowding were less severe under the darkened conditions (Wedemeyer et al. 1985). For this reason, and because of the behavioural avoidance that occurs, taking steps to reduce light intensity during all stages of smolt transport is a recommended practice.

### *Mineral Salt Additions*

The physiological dysfunctions responsible for the immediate and delayed mortality that can occur when fish are transported, and the use of mineral salt additions to improve survival by mitigating these dysfunctions, have received considerable attention in recent years (Johnson and Metcalf 1982; Carmichael et al. 1983, 1984; Wedemeyer et al. 1985). It has long been known that survival rates of transported warm- or coldwater fish could be substantially increased by simply adding sodium chloride (NaCl) at 0.5–1.0% to the hauling tank water (Hattingh et al. 1975; Long et al. 1977). If the fish can be allowed to recover in salt-enriched water after release from the hauling truck, survival is even better (Long et al. 1977; Mazik et al. 1991). Recently, several more complex mineral salt formulations have been developed to mitigate stress and reduce mortality in fish transported in water of low total hardness (Carmichael et al. 1984; Wedemeyer et al. 1985).

The physiological benefits provided by either the simple or complex mineral salt formulations are probably mainly due to the protection they afford against the life-threatening blood electrolyte losses that occur when the diuresis stimulated by handling and crowding stress is prolonged (Wedemeyer 1972; Carmichael et al. 1983, 1984). However, salt formulations can also mitigate other adverse physiological changes, such as the depressed blood pH that results from metabolic acidosis in transported rainbow trout (Haswell et al. 1982), and the hyperglycemia and hypercortisolemia that occurs in transported anadromous salmonids (Wedemeyer et al. 1985). Two disadvantages of mineral salt additives are (1) the potential for uptake of $Mg^{++}$ and $K^{+}$ if scale loss of more than 10% has occurred, and (2) the potential for increased equipment corrosion problems (Wedemeyer et al. 1985). A major advantage of drugs such as MS-222 as transport additives for smolts is the lack of potential for these two problems.

As judged from present experience, single or mixed formulations of NaCl, $Na_2SO_4$, $NaHCO_3$, KCl, $MgSO_4$, $K_3PO_4$, and sea salts, used with or without tranquilizing concentrations of MS-222, have the most potential for alleviating the life-threatening physiological disturbances resulting from hauling smolts or other juvenile salmonids — especially in the slightly acidic waters of low total hardness typical of the west coast and eastern portions of North America. A list of mineral salt formulations recommended to mitigate stress and improve the survival of transported fish is given in Table 3.

### *Polymer Formulations*

The infections that follow abrasion and scale loss due to handling have long been known to be a significant cause of delayed mortality in transported anadromous salmonids and other fish. Frequently, the cause of death is a secondary fungus infection — a common occurrence when even minor skin abrasions disrupt the normal protective slime layer and expose the underlying tissue to attack by *Saprolegnia.* Fungus infections are also an important cause of prespawning mortality in returning adult Pacific salmon, such as the Sacramento River winter chinook, which must be trapped at dams, trucked to a hatchery, and held for extended periods before spawning. The tropical fish industry has successfully used water additives containing polymers such as polyvinylpyrrolidone (PVP) to solve a related problem in transported aquarium fish. When abrasions and scale loss occur, the polymers temporarily bond to the exposed tissue and are gradually sloughed off as healing takes place and the normal slime layer reforms. This technology has received only limited use in warm- and coldwater fish culture where the approach has been to reduce mortality by using transport additives such as mineral salts and anaesthetics that mitigate physiological stress. However, a recent survey has shown that polymer formulations, usually added as one of the commercially available products such

TABLE 3

Mineral salt formulations used for transporting salmonid smolts. Rainbow trout and selected warmwater species are included for comparison.

| Salt formulation | Concentration | Species transported | Reference |
|---|---|---|---|
| NaCl | 0.5-1% | Coho, chinook, steelhead | Long et al. (1977), Wedemeyer et al. (1985) |
| $NaHCO_3$ | 250 ppm | Chinook smolts | Wedemeyer et al. (1985) |
| $CaCl_2$ | 50 ppm | Chinook smolts | Wedemeyer et al. (1985) |
| $CaCl_2$ + $NaHCO_3$ | 50 + 250 ppm | Chinook smolts | Wedemeyer et al. (1985) |
| MS-222 + $NaHCO_3$ | 10 + 250 ppm | Chinook smolts | Wedemeyer et al. (1985) |
| MS-222 + $CaCl_2$ | 10 + 50 ppm | Chinook smolts | Wedemeyer et al. (1985) |
| MS-222 + $CaCl_2$ + $NaHCO_3$ | 10 + 50 + 250 ppm | Chinook smolts | Wedemeyer et al. (1985) |
| $NaHCO_3$, $Na_2SO_4$ | 10 mM | Rainbow trout | Haswell et al. (1982) |
| NaCl | 0.1-1.0% | Rainbow trout | Carmichael and Tomasso (1988) |
| KCl + NaCl + $MgSO_4$ + $K_3PO_4$ | *see* footnote 1 | Largemouth bass | Carmichael et al. (1984) |
| Sea salts | 0.5% | American shad | Backman and Ross (1990) |

[1] Quantity sufficient to be isotonic with blood when added to minerals naturally present in the hauling tank water.

as Polyaqua®, are now finding a certain amount of use by the aquaculture industry and by state and federal conservation hatcheries as a water additive for transporting juvenile salmonids as well as other fish (Carmichael and Tomasso 1988). In the author's experience, a concentration of 100 ppm Polyaqua® during trucking has significantly reduced the prespawning mortality of adult fall chinook salmon and steelhead trout due to *Saprolegnia*. Results were also promising when Polyaqua® was used at 100 ppm in the anaesthetic and holding tanks while examining adult steelhead trout for spawning ripeness. At the U.S. Fish and Wildlife Service Coleman National Fish Hatchery, the prespawning mortality of treated adult steelhead subjected to repeated handling over a 3-month period was about 7% compared with the 30-50% mortality sometimes experienced.

## SPECIFIC TRANSPORT EQUIPMENT AND METHODS

Although a variety of equipment and methods has been employed in the ongoing effort to improve the survival of transported fish, the life support systems required must actually support only two basic types of transportation. These are (1) open systems: continuously aerated tanks carried on trucks, trailers, aircraft, barges or ships, and (2) closed systems: shipments of gametes, fertilized eggs, or fish in sealed containers, usually plastic bags.

### *Open Systems: Continuously Aerated Containers*

*Basic Hauling Tank Design.* Although custom designed vehicles dedicated solely to fish hauling are highly desirable, separate tanks fabricated of aluminum, stainless steel, fibreglass, or wood that can be carried on existing trucks, trailers, ships, or aircraft are more commonly used because of the lower cost (Carmichael and Tomasso 1988). Fish hauling tanks are usually elliptical or rectangular in cross section and insulated with 3-8 cm of polyurethane or styrofoam for temperature control. Elliptical tanks, which are normally fabricated of aluminum or stainless steel, are the most expensive but their centre of gravity is toward the centre of the transport vehicle, they weigh less per unit volume of capacity, and they have a virtually unlimited service life. However, because of the lower initial cost, the great majority of transport tanks in use today are rectangular and constructed of fibreglass, aluminum, or marine grade plywood coated with fibreglass, and then insulated.

The smolt hauling tanks of the U.S. Army Corps of Engineers in which downstream migrant salmon and steelhead smolts are collected and trucked or barged around the dams on the Columbia and Snake Rivers are the largest in current use. The truck- or trailer-mounted elliptical tanks hold 3,500 gallons (132,500 l) of water and are capable of hauling about 1,700 pounds (771 kg) of smolts at a nominal loading density of 6% (0.06 kg/l, 0.5 lb/gal). Larger 150,000 gallon (568,000 l) barge-mounted rectangular tanks use pumped river water together with supplemental oxygenation to haul fish loads of about 75,000 pounds (34,000 kg) at the same weight-loading density (6%), with a flow density of 0.2 gpm/lb of fish (1.7 lpm/kg). Species transported normally include yearling and subyearling chinook, coho, and sockeye salmon, and steelhead trout. In 1990, about 1.5 million smolts were transported by truck and nearly 20 million by barge with very low mortality (Ceballos et al. 1991). Hauling tanks used by state and federal fisheries agencies in routine hatchery operations in the U.S. are generally much smaller, averaging about 500 gallons (1900 l) in size (Carmichael and Tomasso 1988).

In any hauling tank design, the first requirement is the necessity to continuously circulate freshly aerated water to all parts of the container, regardless of size. This is absolutely essential to the health and survival of transported fish and a recirculation rate equivalent to at least 0.5 water exchanges per minute is widely considered desirable for salmonids. In some designs, the hauling tank water is recirculated through overhead spray bars by gasoline-powered centrifugal pumps. Because centrifugal pumps cause compression and frictional heating of as much as 1-3°C per hour, a mechanical refrigeration system is usually also installed to maintain the desired water temperatures.

An alternative circulation system is low voltage electrical agitators (aerators) mounted through holes in the tank surface. These units simultaneously circulate and spray-aerate the hauling tank water without the heating caused by centrifugal pumps and are gradually becoming the dominant design (Carmichael and Tomasso 1988). Exterior foam insulation (5-8 cm) painted white is generally adequate to prevent water temperatures from increasing more than 1°C per 4-5 hours of travel time even in air temperatures of 30-35°C. For longer trips, chlorine-free ice can be added as necessary. One 12-V agitator unit (of the Fresh Flow™ design) per 200 gallons of hauling tank volume will provide a maximum water recirculation rate of about 0.5 exchanges per minute (Figure 3). The 12-V electrical power required can usually be provided simply by replacing the truck's factory alternator with a commercially available 110-A heavy duty unit.

The second requirement in hauling tank design is the necessity to continuously remove the $CO_2$ stripped out of the water by the aeration system into the tank head-space. This feature does not always receive adequate attention, but clearly the efficient stripping and air-equilibration of dissolved $CO_2$ made possible by oxygen aeration and spray agitators will accomplish little unless the $CO_2$ removed from the water is also rapidly removed from the headspace as well. Otherwise, it will simply redissolve. In one study, the respiration of fish held in aerated but covered distribution units caused the dissolved $CO_2$ to increase to 30 mg/l within 90 min. Simply removing the covers allowed the $CO_2$ concentration to decrease to only 5 mg/l with no change in aeration rate or other variable (Smith 1978). Thus, a tank with an open top would provide the most efficient $CO_2$ removal and this design is now being increasingly used to haul species that are not light sensitive. A 6-8-inch rim (15-20 cm) is installed around the sides and bottom edge of the screened opening to prevent water loss from splashing. The open top also allows some evaporative cooling to occur. The traditional closed top fish distribution unit, though not as effective as the newer open top design, must still be used when hauling smolts and other light sensitive fish. Covered tanks require forced ventilation of the headspace to adequately remove $CO_2$ and air scoops or vents are usually mounted on the tank surface to accomplish this.

The third requirement in hauling tank design is the absolute necessity for an adequate aeration system. The importance of this is self-evident. Failure to provide adequate dissolved oxygen to fish results first in hypoxia, then unconsciousness, and eventually death. The aeration system also functions to strip expired $CO_2$ from the water. Failure to adequately remove carbon dioxide results in hypercapnia, respiratory stress from the Bohr and Root effects, $CO_2$ narcosis, and eventually death.

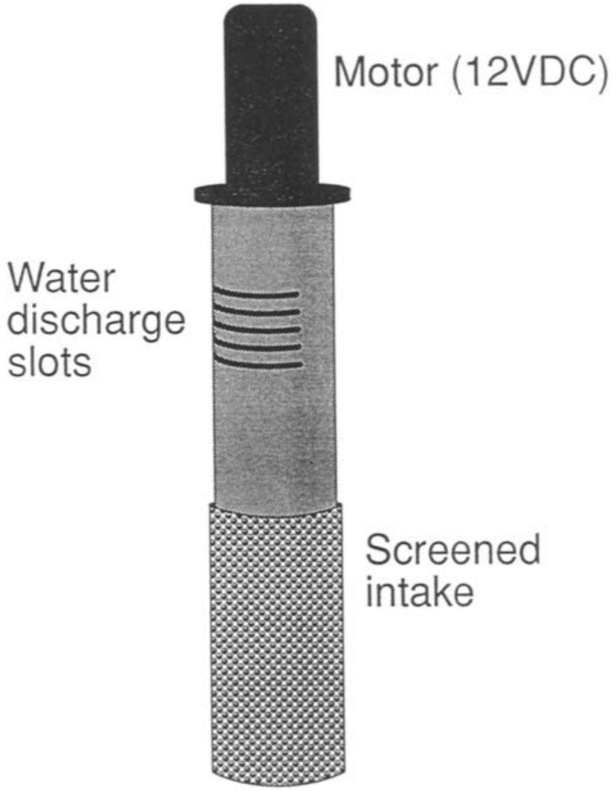

FIGURE 3. An agitator of the Fresh Flow™ design used to recirculate and spray-aerate hauling tank water. The cylindrical, screened intake tube contains the propeller and is sized to reduce intake velocities below the point at which fish impingement would occur.

Although the spraying action provided by either recirculating pumps or agitators results in a small gain in DO, it serves primarily to air-equilibrate the dissolved waste gases and supplemental aeration is always required. This can be provided either by injecting compressed oxygen into the intake side of the recirculating pump, if pumps are used, or into micropore tubing or carbon rod or ceramic diffusers mounted on the tank bottom. Carbon-rod diffusers are the most commonly used. The recommended size is 0.3 m of rod length per lpm of oxygen flow. A drain system is usually included as well to eliminate the need to net fish out of the tank at the end of the trip. If a perforated false bottom is used, the micropore diffusers must be mounted above it or aeration efficiency will be greatly decreased.

Experience has shown that aeration sufficient to provide the minimum 7–8 mg/l DO concentration, often considered satisfactory for hatchery rearing, does not provide an adequate safety margin when transporting fish because metabolism is generally higher and more labile than normal due to excitement and stress. Also, even under resting conditions fish have a very small blood oxygen safety margin. The residual oxygen concentration in the venous blood is typically only about 2 volume percent (v%) compared with about 13 v% in terrestrial animals (Itazawa 1970). Thus, the capacity to provide a DO concentration of at least 80% of saturation in the hauling tank water is now a routine requirement in aeration system design (Westers 1984). As mentioned, pumping the hauling tank water through overhead spray bars or recirculating with agitators of the Fresh Flow™ design serves mainly to air-equilibrate dissolved $CO_2$ and supersaturated gases — only a minor increase in DO occurs. Maintaining the DO at or near saturation with fish loads that are high enough to be practical always requires supplemental aeration — usually injected through gas diffusers mounted above any false bottom on the tank floor. Due to the development of carbon-rod and ceramic micropore diffusers, the efficiency of gas transfer into the hauling tank water has increased to 50% or more. Pure oxygen aeration systems have become economical and are now the dominant design in fish transport systems. As mentioned, oxygen aeration also increases the efficiency of stripping unwanted dissolved gases such as $CO_2$ and $N_2$ from the hauling tank water. As oxygen bubbles from an air stone rise through the tank, $O_2$ diffuses out into the water and dissolved $CO_2$, $N_2$ and other gases diffuse inward and are eliminated. Both processes are driven by the partial pressure differential.

Unfortunately, calculating the oxygen flow rates that will maintain the DO at or near saturation and also adequately strip out unwanted dissolved gases is difficult to do because of the many variables involved. The rate at which fish consume oxygen and produce $CO_2$ depends on factors such as the degree of excitement, swimming activity, loading density in the hauling tank, time since last feeding, transport time, and water chemistry conditions such as temperature, pH, and $CO_2$ concentration (Johnson 1979). The oxygen flow rate required will also depend on factors as disparate as the bubble size produced by the diffusers and the amount of stress caused by the collection and loading procedures (Wedemeyer et al. 1990).

One rationale for the aeration requirements of transported salmonids was suggested by Fry and Norris (1962) who proposed that the aeration rate be sufficient to provide half the oxygen needed by the fish at their maximum swimming activity level. For example, if the maximum oxygen consumption rate of a particular salmonid at 10°C is 350 $mg \cdot kg^{-1} \cdot h^{-1}$ (which can be supplied by a DO of about 12 mg/l), and the resting rate is 75 $mg \cdot kg^{-1} \cdot h^{-1}$; the oxygen consumption rate at the half maximum activity level would be 275 $mg \cdot kg^{-1} \cdot h^{-1}$, which could be supplied by enough aeration to maintain a DO of 6 mg/l. In practice however, this or other aeration calculation methods provide an inadequate safety margin and are not normally used. Other than the generalization that the aeration rate should be kept high enough to provide a margin of safety during the haul, the only minimum usually specified is a rate that will keep the DO at or above 80% of saturation. This translates into a DO of about 10–12 mg/l if the hauling tank water is kept cooled to 10°C. In U.S. Fish and Wildlife Service fish transport operations, the oxygen flow rate guideline for salmonids is 3 lpm/250 kg of fish per metre of carbon-rod diffuser length (i.e., 0.3 m diffuser rod per lpm flow).

One disadvantage of the change to oxygen aeration is that it has now become possible for oxygen supersaturation of as much as 200% to occur in the hauling tank water, raising the possibility of gas bubble disease (GBD). The blood and tissues of fish quickly reach equilibrium with the partial pressures of the gases dissolved in water. If these gases are supersaturated, they readily come out of solution in the blood and tissues and form bubbles that can block circulation to vital organs. Fortunately, when oxygen bubbles form they are usually quickly reduced in size by the metabolic demand of the tissues and mortality from GBD is normally not a problem except at very high oxygen supersaturation levels. The mortality from GBD due to the air supersaturation that can occur as a result of decreased atmospheric pressure during aircraft transport will be discussed in a later section of this chapter.

Although hauling fish in oxygen-supersaturated water (hyperoxia) does provide an economic advantage by allowing the loading density safety margin or the total weight of fish hauled to be increased, there is no real evidence of improved survival or other physiological benefit. One disadvantage is that hyperoxia results in an increase in arterial $CO_2$ pressure. The resulting respiratory acidosis is compensated by an increase in plasma bicarbonate (Hobe et al. 1984). When smolts transported in hyperoxic water are released directly into normoxic seawater net pens, a rapid transfer of the elevated blood $CO_2$ into the seawater occurs that results in a concomitant increase in blood pH. The resulting metabolic alkalosis may require as much as 24 h for compensation. Experience has shown that smolts can become agitated when released directly into salt water from freshwater hauling tanks (Pennell 1991). This may be due to the fact that smolts under these circumstances are simultaneously challenged with increased salinity, lowered blood oxygen levels, and water temperature changes. Although these challenges are normally within the tolerance limits of smolts in good condition, hyperbaric oxygen tensions in the hauling tank water will add blood pH and $CO_2$ alterations to the required physiological adjustments

As previously discussed, the second major purpose of the aeration system is to help prevent $CO_2$ accumulation in the hauling tank water. Rainbow trout transported at the normal loading density of 1.4 kg/l (12 lb/gal), can produce dissolved $CO_2$ concentrations of 30 mg/l or more within 30 min (Smith 1978). The gas displacement and stripping action provided by the oxygen diffuser system helps transfer this expired $CO_2$ from the hauling tank water into the tank headspace. As mentioned, it is important to continuously remove this $CO_2$ from the headspace by forced ventilation or by using a tank of the newer open top design.

*Hauling Tank Carrying Capacity.* The maximum weight of fish that can be routinely transported in an open system fish distribution unit ultimately depends on the interactions of a number of abiotic and biotic variables that in practice are difficult to quantitate. These include the physiological characteristics of the fish to be hauled as well as the efficiency of the aeration system and chemical composition of the water. For example, corneal abrasion and blindness can occur when largemouth bass (*Micropterus salmoides*) are transported at densities that do not otherwise violate guidelines for recommended water quality (Ubels and Edelhauser 1987). Thus, the maximum carrying capacity for a particular species and distribution unit design must usually be determined by experience. In addition, loadings found to be successful can be expected to vary somewhat among hatcheries. Despite these limitations however, useful carrying-capacity guidelines have been developed. For example, the carrying capacity that can be achieved is a direct function of fish size (Westers 1984). That is, a heavier load of large fish than of small fish can generally be transported per unit volume of water. For salmonids, this maximum permissible weight has been found to be directly proportional to fish length. Thus, if experience shows that a particular distribution unit design will safely carry 100 kg of 2 cm trout, it will also be capable of transporting about 200 kg of 4 cm trout; all other factors being equal (Piper et al. 1982).

In practice, the life support systems now available allow hauling densities of about 36% (0.4 kg/l, 3 lb/gal) to be routinely achieved when transporting larger (25 cm) non-anadromous rainbow trout, or brown (*Salmo trutta*), brook and lake (*Salvelinus namaycush*) trout (Piper et al. 1982; Westers 1984). For anadromous salmonids, carrying capacities in the range of 6-24% (0.06-0.24 kg/l, 0.5-2.0 lb/gal) for fingerlings and 24-36% (0.24-0.36 kg/l, 2.0-3.0 lb/gal) for the larger parr (10-12 cm) are easily attained. In smolt hauling, considerably lower loading densities are recommended because anadromous salmonids during this life stage are more sensitive to handling and crowding stress and scale loss (Barton and Iwama 1991). In trucking smolts, the (conservative) guideline widely used in the U.S. is a weight loading of 6% (0.06 kg/l, 0.5 lb/gal). In barges or ships using pumped river or ocean water, the same weight loading with a flow rate of about 2 lpm/kg of fish (0.2 gpm/lb) is used to achieve an adequate tank circulation rate (Ceballos et al. 1991).

A complicating factor in determining and reporting the carrying capacity of open systems is that fish density is commonly expressed in various ways such as percent loading (weight of the fish as a percentage of the weight of the water), weight loading (weight of fish per unit volume of water), or as the displaced weight loading (weight of fish per unit volume of water minus the water displaced by the fish). In calculating loading densities by the latter method, the volume of the water displaced (tabulated for convenience in Table 4 for non-metric system users) is subtracted from the tank volume as it is not available to the fish. The loading density (LD) is then:

$$\text{LD} = \text{fish weight}/(\text{water volume} - \text{water displaced by the fish}) \qquad (1)$$

TABLE 4

Volume of hauling tank water displaced by a given weight of fish. Based on an average value for the specific gravity of teleosts of 1.0 (McCraren and Jones 1978).

| Fish weight | | Water displaced | |
|---|---|---|---|
| **Pounds** | **Kilograms** | **Gallons** | **Litres** |
| 200 | 91 | 24 | 91 |
| 400 | 181 | 48 | 182 |
| 600 | 272 | 72 | 273 |
| 800 | 363 | 96 | 363 |
| 1,000 | 454 | 120 | 454 |
| 1,200 | 544 | 144 | 545 |
| 1,400 | 635 | 168 | 636 |
| 1,800 | 816 | 216 | 818 |

Using this method of calculation, the loading density of 800 lb of fish added to 500 gal of water in a tank would be:

$$LD = 800/(500 - 96) \approx 2.0 \text{ lb/gal } (24\%, 0.24 \text{ kg/l}) \qquad (2)$$

If the water displaced by the fish is not subtracted, the loading density would appear to be 800/500 = 1.6 lb/gal (19%, 0.19kg/l), a substantially lower figure. The water displacement method of expressing carrying capacity has been recommended by Piper et al. (1982), but any method can be used provided it is used consistently.

*Disinfection Methods.* Hauling tanks of any design must be cleaned and disinfected on a routine basis to prevent the transmission of fish diseases. Chlorine at a concentration of 100-200 mg/l with a contact time of at least 30 min is the most commonly recommended disinfecting agent (Leitritz and Lewis 1976). Of necessity, disinfection protocols will vary somewhat but in general, sufficient water to cover the intakes of the recirculating pumps or spray agitators is poured in, the calculated amount of liquid or dry chlorine is added (e.g., 15 g HTH, 70% available chlorine, per 100 l), and the solution is pumped through the system for about an hour. Air or oxygen must be flowing through the diffusers during this time. If the water is alkaline, sufficient glacial acetic acid should be added to decrease the pH to about 6 in order to activate the chlorine. After disinfection, the chlorine is neutralized by adding sufficient sodium thiosulphate to achieve a concentration of about 700 mg/l, and the solution is discharged in an appropriate location. As a precaution, neutralization should be confirmed with commercially available chlorine test paper and the tank thoroughly flushed with chlorine-free water before loading fish.

*Transport by Aircraft.* Experience has shown that transporting fish with fixed-wing aircraft or helicopters can be very cost effective in some circumstances and thus it has now become a relatively common practice (Shepherd and Bérézay 1987). Fixed-wing aircraft have traditionally been used both to stock remote lakes and streams by air drop and to haul smolts to ocean net-pen sites. However fish survival following air drop has been shown to be lower than with other forms of transport (Fraser and Beamish 1969) and smolt hauling has now become the predominate use.

In transporting smolts to remote sea pen sites by float plane, custom designed tanks are sometimes used, but garbage cans lined with appropriately sized plastic bags are more often pressed into service. The bags are filled with oxygenated fresh water with or without added ice, depending on the length of the flight and the temperature differential between the freshwater hatchery and the sea pens. As with other forms of transport, salt (NaCl) at 0.1% is often added to mitigate stress. Generally, the smolts are loaded into the hauling containers at a density of 12-16% (0.12-0.16 kg/l). Loading densities at the lower end of the range are used for smaller fish. The cans are loaded onto the aircraft (usually a single or twin engine float plane), and the DO is adjusted and maintained at 10-12 mg/l with compressed oxygen during the flight. A backup supply of ice and oxygen is normally carried aboard the aircraft. During a typical flight of about 45 minutes, the water temperature of the cans may rise a few degrees. Upon arrival, the plastic bags containing the smolts are removed and floated in the sea pens allowing the temperature, salinity, and DO to gradually equalize.

Helicopter transport, although more expensive on a per hour basis, allows greater flexibility and has now become the dominant method of hauling smolts by air (Shepherd and Bérézay 1987). Commercially available water carrying buckets designed for fire-fighting can be easily modified for fish hauling and so are frequently employed as transport containers. The total weight of fish and water carried is determined by the lifting capacity of the helicopter used — generally about 400 kg (900 lbs). Several bucket designs are available: rigid-wall fibreglass or aluminum, collapsible fabric with a metal frame, or an aluminum container with individual bottom dumping compartments, each separately hinged and opened by solenoids controlled from inside the helicopter (Larson 1984). The buckets are flown to the marine sites suspended from the helicopter on a long cable. Aeration is normally accomplished with a small compressed oxygen tank strapped to the bucket and connected to an airstone or micropore tube secured to the bottom of the tank. A clear plastic lid locked on the bucket will minimize the chance of accidental dumps, reduce water loss, and allow the pilot to visually check the fish during the flight. If there is no ground crew at the release site, a bucket with a tapered bottom section will aid the pilot in overturning the hauling container to ensure that all fish are released (Shepherd and Bérézay 1987). A smolt transport tank has also been developed that can be carried inside a helicopter. Safety problems inherent to buckets suspended by cables are eliminated and costs are reduced from the normal 5-6¢ per smolt to 3-4¢ (Black et al. 1991).

Air supersaturation with resultant mortality from gas bubble disease due to the rapid reduction in atmospheric pressure as the helicopter climbs to its normal cruising altitude can be a serious problem in salmonid fry and fingerling transport operations (Hauck 1986). At altitudes above about 0.6 km (2,000 ft), the atmospheric pressure reduction is sufficient to cause the hauling tank water to exceed 110% of air saturation. This can result in gas bubbles in the fins, eyes, and yolk sac of transported fry. Precautions to be taken include chilling the hauling tank water to prevent the additional supersaturation caused by water temperature increases, minimizing the rate of ascent, and flying at the lowest cruising altitude consistent with safety. If the stocking site is at sea level, recompression during the descent may be sufficient to reverse the bubble formation process and prevent mortalities. Fortunately, larger fish are generally less susceptible and GBD has not been reported to be a problem during aircraft transport of smolts.

*Transport by Ships or Barges.* Transporting smolts in specially designed tanks on ships or barges can be quite practical under certain conditions. Live-well tanks on ships are sometimes used by the aquaculture industry when long-distance transfers of smolts or adults are necessary. Tank capacities are typically 60,000 l (16,000 gal) or more, and smolt hauling operations from hatcheries as well as farm-to-farm transfers of larger fish are carried out. Well-boats using flow through seawater tanks with supplemental oxygen or compressed air aeration, and closed circuit television cameras to visually check the condition of the fish have been successfully used to transport smolts at loading densities of 0.4–0.7% (4–7 kg/m$^3$) over distances of 1000 km (700 miles) or more. A major precaution required is to avoid areas of toxic marine algae blooms (Pennell 1991).

Barges are being used extensively in the U.S. to transport migrating juvenile Pacific salmon and steelhead trout around the dams on the Columbia and Snake rivers in what has become the largest smolt hauling operation in the world. Flow-through tanks as large as 570,000 l (150,000 gal) are used with river water circulated by centrifugal pumps through overhead spray bars to dissipate supersaturated gases. Compression heating is of no concern in this application. Liquid oxygen is used for supplemental aeration. A fleet of six barges is used, sometimes operating around the clock. Loaded at densities of 6% (0.06 kg/l, 0.5 lb/gal) with a circulation rate of about 2 lpm inflow of pumped river water per kilogram of fish, the carrying capacity of a single barge is about 34,000 kg (75,000 lb) of smolts. In some years, 20 million or more migrating juvenile coho, chinook, sockeye salmon, and steelhead trout are collected and transported around as many as eight dams and nearly 500 km of impounded river (Ceballos et al. 1991).

### *Closed Systems: Shipments in Sealed Containers*

Gametes, fertilized eggs, alevins, and other small fish held in oxygenated closed containers cooled with crushed ice can easily be transported long distances. Tropical and warmwater fish, as well as juvenile salmonids, are routinely shipped around the world in this manner (Stickney 1983).

*Gametes, Fertilized Eggs.* Salmonid eggs are normally transported during three stages of their development: as unfertilized eggs (gametes); as newly fertilized (green) eggs for about 12 h after water hardening; and at the eyed stage (after the embryo has developed eye pigmentation).

Gametes are infrequently transported. When it is necessary, the sperm is shipped separately in a sealed container (usually a plastic bag) on ice. An air space of about 10 volumes of air to 1 volume of sperm is required in the container to provide a sufficient amount of oxygen. The unfertilized eggs are also shipped in sealed containers on ice but will suffer high losses if shaken or jarred in transit. To minimize movement, the containers are completely filled with eggs — no air space is allowed. If the temperature is not allowed to rise above 2-5°C, the fertility of the transported sperm and eggs will remain high for at least 4-5 h (Banks and Fowler 1982).

Green (newly fertilized) eggs can be shipped during the period of about 12 hours after water hardening but their initial sensitivity to mechanical shock begins to develop as soon as 15 min after fertilization, and precautions to prevent jarring or movement are absolutely essential (Banks and Fowler 1982). Shipping the eggs totally submersed in water with no air space in the container will help minimize losses. During the period from about 48 h after fertilization until eye pigmentation has developed (eyed stage), incubating eggs should not be disturbed for any reason.

After the embryo has developed to the eyed stage, eggs become resistant to mechanical shock and can be shipped out of water over long distances if kept moist and cool. Sealed shipping containers that provide and maintain the required environmental conditions have been designed and eyed eggs are now routinely transported around the world. The containers are commercially available and usually consist of an insulated box with stacked perforated trays arranged to keep the eggs moist and cool without actually immersing them in water. Wet cheese cloth may or may not be placed on the bottom of each perforated tray before the eyed eggs are poured in. If cheese cloth is used, it is then folded over the eggs and the trays are stacked vertically in the box. The top tray is filled with cubed or crushed ice to cool the eggs during shipping and to help retard hatching. As the ice melts, the cold water drips down through the perforated trays and over the eggs to keep them moist, facilitate gas exchange, and wash away toxic metabolites. To prevent cold shock, ice taken directly from a freezer should never be used until it has warmed enough to begin to melt (Leitritz and Lewis 1976).

*Alevins, Juvenile Fish.* Small lots of alevins and other small fish can also be transported successfully in closed systems. Plastic bags partially filled with oxygenated water with an oxygen atmosphere are generally used. For fingerling salmonids, the accepted loading density is 50–100 g/l, with an air:water ratio of at least 3:1, for trips of 12 to 48 h (Shepherd and Bérézay 1987). Loading densities for other species and sizes are given in Table 5.

A number of successful protocols have been developed. One method which allows up to 500 1-2 g salmonids to easily withstand shipping times of 10 h or more employs milk dispenser bags commonly used in the dairy industry. The bags are made of double-walled food grade polyethylene, have a neoprene plug with a short length of self-sealing tubing attached, and are inexpensive. A bag holder is also commercially available.

In this shipping method, the plastic bag is placed on the bag holder, the plug is removed and about 1 kg (2.2 lb) of crushed chlorine-free ice is added through a funnel. About 10 l of $O_2$-saturated chilled water is added followed by up to 500 1–2 g fingerling salmonids previously acclimated to 5°C. The bag now containing fish, ice, and water is removed from the holder, the air squeezed out and the plug replaced. A hypodermic needle attached to an oxygen line is inserted into the self-sealing tubing and the bag partially inflated. A suitable shipping container is selected, such as an insulated plastic lined cardboard box or plastic bucket with tight fitting lid. The bag is placed inside and more ice is packed around it for additional cooling. If plastic buckets are used, the additional cooling can be achieved by adding water and freezing it before the plastic bag is inserted.

At the normal (5–10%) loading densities used, salmonid fish can be held in the cooled and oxygenated plastic bags for about 10–12 h before $CO_2$ and ammonia begin to approach toxic levels. This time can be significantly extended by adding a small amount of zeolite or other commercially available ion exchanger to absorb the ammonia (e.g., clinoptilolite at 14 g/l), and tris buffer (0.02 M, pH 8) to prevent metabolic acidosis from the expired $CO_2$ (Amend et al. 1982*a*; Stickney 1983).

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TABLE 5

Loading densities (g/l) acceptable for shipping warmwater and coldwater fish in cooled, sealed containers with an oxygen atmosphere (Johnson 1979).

| **Type of fish** | **Shipping time (hours)** | | | |
|---|---|---|---|---|
| | **1** | **12** | **24** | **48** |
| Ictalurid catfish | | | | |
| fingerlings (8 cm) | 100 | 75 | 50 | 25 |
| fry (0.6 cm) | 50 | 40 | 30 | — |
| Aquarium species (2-5 cm) | 100 | 75 | 50 | 25 |
| Salmonids (1-2 g) | 100 | 100 | 50 | 50 |

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## Chapter 13

# SALMONID DISEASE MANAGEMENT

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## INTRODUCTION

### *Scope of Chapter*

Diseases play an important role in salmonid cultivation and are frequently the most important profit-limiting factor in intensive aquaculture. The potential effects of diseases can be economically disastrous and farmers cannot afford to be unaware of the importance of disease control and management practices. This chapter provides some examples of the importance of diseases in salmonid cultivation and an historical overview of the development of scientific understanding of fish disease control and management. This is followed by an overview of the infectious diseases of salmonids (viral, bacterial, fungal and parasitic), with emphasis on the most economically important diseases. There is a huge body of information available on this subject and it is beyond the scope of this chapter to provide extensive details concerning these pathogens and their pathological effects. For such information the reader is referred to other specialized texts (Roberts 1982, 1989; Austin and Austin 1987; Wolf 1988; Inglis et al. 1993*b*; Stoskopf 1993).

In the first of the next two sections of the chapter, the techniques used to recognize and diagnose disease in salmonids are described. Some of these techniques are simple and can be used to good effect by farmers on site, while at the other extreme, some diagnostic techniques can be very sophisticated requiring well equipped laboratories and specialized personnel to perform them. The next section concentrates on the management aspects of disease control and emphasizes the central role of good husbandry in salmonid cultivation. Many integral aspects of salmonid cultivation are unavoidably stressful to the fish but an enlightened management can reduce the stress incurred so that the risks of precipitating disease outbreaks can be minimized, thereby ensuring healthy fish and a productive industry.

### *Importance of Disease in Salmonid Culture Programs*

There are many examples of highly pathogenic organisms having devastated salmonid culture programs. There are also countless examples of farms being forced out of business because of poor husbandry practice, resulting in heavy losses from opportunistic pathogens normally present in the aquatic environment. Some examples of heavy losses to whole industries may be cited here. The U.S. Trout Farmers Board of Directors Survey in 1991 indicated that 52% (35.8 million fish of a total of 68.9 million trout produced) died as a result of disease. Most of these fish died as fingerlings and the greatest single cause is thought to be from infectious haematopoietic necrosis (IHN) (Leong and Fryer 1993). In Europe losses in trout farms from viral haemorrhagic septicaemia (VHS) have been estimated to amount to $80 million per year (Hill 1991).

During the late 1980s, losses attributed to furunculosis in the Scottish Atlantic salmon (*Salmo salar*) sea-cage industry were estimated to be about $30 million per year. From seawater entry to harvest more than 30% of the stock were lost and much of this was attributed to furunculosis (Munro and Hastings 1993). In the Pacific Northwest of North America, bacterial kidney disease has, for many years, caused significant losses in the culture of Pacific salmon species, and has been a major restricting factor on culture of these species (Fryer and Lannan 1993). Soft flesh induced by the protozoan *Kudoa* in Atlantic salmon in British Columbia resulted in claims by the industry of $350,000 in 1993 (Needham 1994). Plasmacytoid leukaemia (PL) in pen-reared chinook salmon (*Oncorhynchus tshawwytscha*) has been a significant problem in western British Columbia aquaculture since 1988 (Kent et al. 1990). The B.C. Ministry of Agriculture and Fisheries estimates losses worth up to $20 million annually. Eaton and Kent (1992) showed the disease was likely due to a new retrovirus, called the salmon leukaemia virus (SLV).

### *Historical Overview and Development of Scientific Understanding of Fish Diseases*

The science of fish diseases has developed relatively recently in response to the rapid growth of the fish farming industry over the last three decades. However, the importance of fish diseases was recognized much earlier in connection with hatcheries in the U.S., U.K. and northern Europe that provided fish, principally salmonids, for stocking sport fisheries during the end of the 19th century. The science began with the bacteriological isolation of *Aeromonas salmonicida* in 1894 (Emmerich and Weibel 1894) and recognition of the importance of fish diseases to wild salmon stocks culminated in the reports of the Furunculosis Committee commissioned by the U.K. Government in 1929 and published between 1933 and 1935 (Mackie et al. 1935). Besides providing important insights into many aspects of fish diseases and their control, the Committee made recommendations for legislation and control measures. The Committee recognized that the international trading in salmonid eggs and live fish for sport fisheries was capable of disseminating many economically important diseases including furunculosis. The legislation proposed that: (1) owners of wild fisheries should be obliged to report cases of furunculosis to appropriate fishing authorities; (2) no transference of fish from an infected watershed be made until permission has been given; (3) importation of all live fish of the family Salmonidae be prohibited; and (4) provision be made for the necessary bacteriological services at a central laboratory. These recommendations for legislative control of furunculosis formed the basis of the Diseases of Fish Act (1937) and was the first legislative measure taken by any country to control the spread of fish diseases.

The realization that certain diseases like furunculosis in wild stocks could be carried by contaminated eggs led to the development of disinfection procedures. Later, it was discovered for other diseases (e.g., infectious pancreatic necrosis, IPN) that disinfection was only partially effective and even more recently, it was discovered that the bacterium causing bacterial kidney disease (BKD) could be carried within the eggs of salmonids and was thereby resistant to surface disinfection procedures (Bruno and Munro 1986; Evelyn et al. 1986). Thus, concern began to grow within the fisheries industry in the early part of the 20th century that diseases could be imported via contaminated eggs and live fish for stocking purposes.

The development of sport fisheries required rearing of young salmonids and further understanding of the fish's physiology. For instance, an understanding of the effects of environmental factors on gill function and osmoregulation has been essential in the planning of salmonid hatcheries and farms with regard to targeting the minimal basic water quality in the facility. Monitoring these water quality parameters allows the fish disease diagnostician to assess many aspects of fish husbandry. Data on nutritional requirements and digestion physiology have allowed the development of artificial diets; endocrine studies have promoted farmers to manage their own brood stocks; and immunological studies have supported the development of vaccines. These studies of salmonid physiology have been essential in developing the sciences of fish disease epizootiology, diagnosis, pathology and disease management.

During the late 1940s and 1950s, the discovery of sulphonamides and antibiotics lent a boost to controlling bacterial diseases in salmon (Snieszko 1953) in the increasing number of hatcheries in North America and Europe. Experiments on vaccination began in 1942 but did not become commercially attractive or successful until the 1970s (Ellis 1988). From 1950-1970 a great many infectious diseases of fish were documented. The causative agents, mainly bacterial, were characterized, the pathologic consequences described, and chemotherapeutic measures introduced. Methods for diagnosing fish diseases became more precise and standardized. Snieszko (1972) discussed, in detail, the progress made in these areas.

With the almost explosive growth of salmonid farming in the 1960s and 1970s, research in fish diseases attained a heightened importance. With the intensification of fish husbandry methods the risk of epizootic outbreaks increased and disease problems became potentially, and for some farms actually, disastrous economically. Furthermore, greater activity in international trading occurred with the risk of disseminating diseases internationally. Many countries enacted legislative control measures but, in addition, a body of scientific experts was required to carry out diagnosis, assess the impact of disease on the industry, develop new means of controlling the spread and outbreaks of disease, and offer advice to the industry and to governments for administration of the Acts. Today diagnosticians work hand-in-hand with epidemiologists, microbiologists, pathologists, biochemists and immunologists. In recent years these have been joined by molecular biologists whose work is revolutionising diagnostic techniques, epidemiological studies and production of a whole new range of vaccines.

### *Approaches to Fish Disease Management*

The early studies on fish diseases, especially the reports of the Furunculosis Committee, clearly introduced the concept that environmental factors significantly influence the risk of outbreak and severity of disease. This concept was developing throughout animal and human epidemiology as stated by workers such as Gowen (1952): "A clinically manifest disease only results when proper combination of the genotype of the victim, and the genotype of the pathogen, where one is necessary, are properly synchronized with the environment". In the fish disease literature, this principle was emphasized by Snieszko (1972) and is the most important principle in the control and management of fish diseases in the setting of the farm itself.

## VIRAL DISEASES OF SALMONIDS

### *Biology and Classification of the Main Groups Significant to Salmonid Culture*

There are three major viral diseases important in salmonid cultivation: infectious pancreatic necrosis (IPN), viral haemorrhagic septicaemia (VHS), and infectious haematopoietic necrosis (IHN). All three diseases are mainly a problem in rainbow trout (*Oncorhynchus mykiss*) culture in fresh water. Two other diseases of great economic importance to Atlantic salmon culture in seawater in the British Isles and Norway are pancreas disease (PD) and infectious salmon anaemia (ISA), respectively. The aetiological agents of these diseases have not yet been identified but the evidence is strongly indicative of a virus cause. The classification of viruses causing disease in salmonids is shown in Table 1.

**TABLE 1**

Classification of viral diseases of salmonids.

| | Status* | Host | Geographic range |
|---|---|---|---|
| **RNA Viruses** | | | |
| Birnavirus: Infective pancreatic necrosis virus (IPNV) | 9 serotypes isolated:<br>4 European,<br>5 N. American | Fry: rainbow trout, Atlantic salmon, brook trout | Ubiquitous except Australasia |
| Rhabdoviruses: Viral haemorrhagic septicaemia virus (VHSV) | Isolated 4 serotypes | Rainbow trout fry and adults | Mainland Europe & N. America |
| Infectious haematopoietic necrosis virus (IHNV) | Isolated 5 serotypes | All salmonid fry and juveniles | N. Pacific rim and mainland Europe |
| Retrovirus: Plasmacytoid leukaemia | EM | Chinook salmon | British Columbia |
| **DNA Viruses** | | | |
| Herpesvirus: Herpesvirus salmonis | Isolated from ovarian fluid | Rainbow trout; no disease symptoms | North America |

**TABLE 1 cont'd.**

| | Status* | Host | Geographic range |
|---|---|---|---|
| **DNA Viruses cont'd.** | | | |
| *Oncorhynchus masou* virus | | Pacific salmon | Japan |
| Iridovirus: Erythrocytic necrosis virus (ENV) | EM | Most salmonids | Atlantic rim |
| Epizootic haematopoietic necrosis virus (EHNV) | Isolated | Rainbow trout | Australia |
| Untyped viruses: Papilloma virus | EM | Atlantic salmon parr | Europe |
| Erythrocytic inclusion body syndrome (EIBS) | EM | Chinook, coho, Atlantic salmon, rainbow trout | NW America, Norway |
| Suspect viruses: Infectious salmon anaemia (ISA) | EM | Atlantic salmon (seawater) | Norway |
| Pancreas disease (PD) | Not observed | Atlantic salmon (seawater) | Europe |

*isolated = cultured in tissue culture EM = observed by electron microscopy

## *Major Viral Diseases*

### *Infectious Pancreatic Necrosis (IPN).*

*1) Modes of expression in fish.* IPN is principally a disease of rainbow trout, brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*) and Atlantic salmon fry. The shipping of contaminated salmonid stock, particularly eyed rainbow trout eggs, has led to a very wide distribution of IPN virus (IPNV). Vigilance by the Australian and New Zealand authorities has so far avoided introduction into these countries. Affected fish display corkscrew swimming, anorexia, darkening, swollen abdomen and trailing faecal casts. The latter is due to a catarrhal enteritis involving a sloughing of the gut lining. The major histological lesion is a pancreatitis (Yasutake 1970; Smail et al. 1992). Significant mortalities can occur from clinical outbreaks in fry. Surviving fish carry the virus, possibly for life, but the virus does not generally cause serious effects in older fish. Larger amounts of virus are shed by clinically ill fish and horizontal transmission and spread of disease through the tank water is rapid. The carrier fish may also shed virus into the water, and such fish are a particular hazard if used as brood stock since the virus can be vertically transmitted either in or on the ova. This is probably the main means of spread of the disease in salmonid culture.

*2) Methods of prevention and control.* The most effective and economical measure to control IPN is by preventing contact between the host and the virus, but this requires the hatchery, its water supply and its source of eggs to be virus-free. Often this means the brood stock must be regularly tested for IPNV and infected fish or eggs destroyed. In commercial operations where IPN cannot be avoided, the vulnerable fry may be reared under virus-free conditions and moved to exposed situations after they have reached a more resistant age.

IPNV is susceptible to iodophore disinfection and various attempts to disinfect contaminated eggs have been made. However, while this may reduce the level of virus, it is not completely effective because the virus is probably within ova as well as on their surface (Dorson and Torchy 1985). A combined approach with the testing for virus-free brood stock, rejection of IPN positive eggs, and iodophore treatment of all ova at stripping is a preferred control strategy.

Eradication by destocking and disinfecting the hatchery premises is recommended when the facility is not at risk from recontamination through its water or ova supply. The virus is quite resistant to ultraviolet light so UV treatment of contaminated water supplies is not effective. A number of attempts to treat infected fry by feeding iodine-compounds and certain antiviral compounds have not been commercially effective.

Injection of high doses of formalin-inactivated virus induce viral neutralising antibodies which can protect salmonids. However, the cost of producing the virus in tissue culture and of injecting very small fry preclude this method of vaccination. Various attempts to develop an attenuated live vaccine that could be administered in the water and cause asymptomatic infections which spread through the population have so far failed (Leong and Fryer 1993). Attenuated strains of IPNV appear to lose the antigens required to induce antibodies with the capability of neutralising the wild-type virulent viruses. However recombinant subunit vaccines are being developed by several groups and progress is encouraging (Manning and Leong 1990; Leong and Fryer 1993).

*Viral Haemorrhagic Septicaemia (VHS).*

*1) Modes of expression in fish.* VHSV has caused serious losses in rainbow trout farms throughout European countries. It has recently been isolated from coho (*Oncorhynchus kisutch*) and chinook salmon, and rainbow trout in North America (Eaton et al. 1991; Hill 1991). Despite policies designed to avoid this disease, VHS virus (VHSV) is probably endemic in affected areas and may be carried by wild and feral salmonids and other freshwater fish species. Transmission is lateral through the water reputedly gaining access to the fish through the gill lamellae. Experimental transmission of VHSV from rainbow trout to pike (*Esox lucius*) and pike to rainbow trout has been demonstrated (Enzmann et al. 1993). There is no conclusive evidence for vertical transmission. Sources of the virus are from carrier fish and introduction with contaminated stock. Fish-eating birds like herons may act as vectors.

This rhabdovirus affects rainbow trout of all ages. The classical symptoms include spiral swimming, darkened skin, exophthalmia and pale gills. Internally, haemorrhages in the musculature, swollen grey-coloured nodular kidney and pale liver may be observed. Mortality rates can be high. In surviving fish the virus may persist in many organs for a duration which is inversely proportional to the temperature. The virus can enter the brain of trout and as antibodies do not gain access to this tissue, the virus may be carried there for long periods of time.

*2) Methods of prevention and control.* Eradication and avoidance has been successfully applied in Brittany and parts of Denmark and Italy. Systematic programs of hatchery disinfection, combined with restocking with specific pathogen-free fish and eggs has been used with considerable success (Enzmann 1983). VHSV is sensitive to chloroform, formalin, iodophores and ultraviolet light. Vaccination with inactivated virus is effective, but culture of the virus is too expensive to develop a commercial vaccine. Recently, progress has been made with a recombinant vaccine produced by cloning the viral gene for the capsid protein into *Escherichia coli* (Lorenzen et al. 1993). This is a cheaper means of mass producing viral antigens and if further tests are successful it should be possible to produce a VHSV vaccine commercially.

*Infectious Haematopoietic Necrosis (IHN).*

*1) Modes of expression in fish.* IHN is a serious disease of salmonids, including Atlantic salmon in hatcheries on rivers flowing to the Pacific coast of North America, especially the Columbia River, and in hatcheries in central and eastern U.S., due to the transport of infected fish or eggs. It has also been reported in Japan and recently isolated outbreaks have occurred in rainbow trout farms in Germany, France and Italy

(Arkush et al. 1989; Leong and Fryer 1993). Most North American salmonid species of all ages can be affected although coho salmon are considered to be quite resistant. A carrier state may be established in survivors, which perpetuate the disease either by vertical transmission within the ova or by horizontal transmission (Traxler et al. 1993).

Affected fish are lethargic and exhibit sporadic whirling. External signs include swelling of the abdomen, exophthalmia, pale gills, haemorrhages at the base of the fins, dark coloration and trailing faecal casts. Internally the kidney, liver and spleen are pale, the stomach and intestine are filled with a milky yellow fluid, and focal haemorrhages may be present in the caeca and adipose tissue and in the body musculature. As the name implies, in moribund fish there is marked necrosis of the kidney and spleen leading to anaemia. Congestion of renal sinuses is considered to lead to death from renal failure. In diseased fry, virus can be isolated from all internal organs and in mature carrier fish virus can be isolated in high titre from gonadal fluids of both male and females.

Water temperature has a strong influence on IHN. The highest mortalities occur at 10°C. Below 10°C the disease is chronic. Above 10°C, while the disease signs are more acute, the mortalities are lower. Outbreaks do not appear to occur above 15°C.

*2) Methods of prevention and control.* A major problem in endemic areas is that the IHN virus (IHNV) is present in wild stocks, and hatchery stocks sharing the same waters are at risk of infection. In non-endemic areas, testing of brood fish and elimination of carrier fish and contaminated eggs are useful in limiting spread of infection. Raising the water temperature to 14°C has been successful in reducing losses in chinook salmon fry in hatcheries but most strains of the virus require a higher temperature to inhibit them.

Protective immunity can be induced by vaccination either by immersion or intraperitoneal injection, but potential commercial development requires gene-cloning technology. Most advances so far in this area have been made with a recombinant subunit vaccine based on the IHNV capsid glycoprotein expressed in *E. coli*, which has been shown to be effective when delivered by immersion in field trials (Leong and Fryer 1993). Further field trials must be carried out, and evidence that this subunit vaccine is effective against all five known serotypes of IHNV in all susceptible salmon species may be necessary before the vaccine can be licensed for commercial use. Resistance to the disease can be heritable and enhanced by selective breeding (McIntyre and Amend 1978).

### *Minor Viral Diseases*

*Oncorhynchus masou Virus*. *Oncorhynchus masou* virus is a herpesvirus that infects salmon belonging to the genus *Oncorhynchus* in Japan. Fish in fresh water are affected and appear sluggish, dark in colour, exophthalmic and may congregate at the water inlet. There is generally no food in the digestive tract. Both splenomegaly and a mottled liver are evident and these organs become white in colour in advanced cases. Histologically the renal tubules and haemopoietic tissues may show necrosis accompanied by pyknosis. Necrosis of the epithelial cells around the head and mouth regions progresses into perioral epithelial neoplasms characterized as papillomas with abnormally proliferating epithelial basal-like cells (Atsuta et al. 1990).

*Viral Erythrocytic Necrosis (VEN).* Viral erythrocytic necrosis (VEN) is an infection of fish erythrocytes caused by the DNA-virus erythrocytic necrosis virus (ENV). It is characterized by cytoplasmic inclusion bodies within erythrocytes. These bodies stain pink by Giemsa and Wright's stain. This infection may cause problems in pink salmon (*Oncorhynchus gorbuscha*) and in chum salmon (*O. keta*) where severe anaemia (Haney et al. 1992) can result in low oxygen stress with increased susceptibility to other diseases, particularly vibriosis in sea water.

VEN has been reported from many parts of the world in most species of salmonids. As affected blood is infectious, the most practical method of control is to avoid close cohabitation between infected and non-infected stock and to inactivate the virus in water before disposal from slaughter units.

*Erythrocytic Inclusion Body Syndrome (EIBS).* Erythrocytic inclusion body syndrome (EIBS) is one of a number of erythrocytic infections similar to VEN that have been reported in coho and chinook salmon and rainbow trout in western North America and in Atlantic salmon in Norway (Lunder et al. 1990). They differ from VEN in that the inclusion bodies are smaller, but the haemolytic anaemia may cause similar effects as VEN. Affected fish are compromised by this anaemia and therefore susceptible to secondary bacterial and fungal infections.

*Plasmacytoid Leukaemia.* A marine anaemia (plasmacytoid leukaemia) has been reported among chinook salmon in sea net-pen farms in British Columbia, Canada. Significant mortalities have occurred among production stock. The disease is characterized by pale gills, splenomegaly and exophthalmos. Histologically there is a massive infiltration and proliferation of plasma cells in the visceral organs. Recent work indicates that an oncogenic retrovirus is the cause of the disease (Kent et al. 1991).

*Atlantic Salmon Papilloma.* This benign papilloma appears as a greyish raised wart on the skin, measuring up 4 cm in diameter and 2-5 mm in thickness. Outbreaks usually occur among salmon in fresh water as single or a multiple proliferation of epithelial cells which may be observed to contain virus-like particles by electron microscopy. Although retrovirus-like particles have occasionally been observed by electron microscopy, the virus has not been cultured and is only presumed to cause this disease. No internal involvement has been found.

The papilloma occurs mainly in Atlantic salmon S2 parr in fresh water where the prevalence may reach 55%. It is rarely seen in marine fish. Outbreaks typically start in

July, peak in August and disappear in December, involving sloughing of the papilloma and healing of the residual ulcer. The main danger of this disease is the possible colonisation of the ulcer by secondary bacteria or *Saprolegnia* fungus (Bylund et al. 1980).

*Herpesvirus Salmonis (HPV).* Herpesvirus salmonis (HPV) is a symptomless infection that has been reported mainly from rainbow trout in North America. HPV has been isolated from ovarian fluid of apparently normal brood fish (Wolf 1976), but laboratory experiments indicated it was pathogenic to rainbow trout (Wolf and Smith 1981).

*Epizootic Haematopoietic Necrosis (EHN).* Epizootic haematopoietic necrosis (EHN) is a minor disease of cultured rainbow trout in Australia (Langdon et al. 1988) and is the only viral disease of salmonids in that country. Affected fish are darkened, lethargic and anorexic. The kidney and spleen may be swollen and pale. Mortality rates were shown to be low but morbidity rates very high.

### ***Suspected Virus Diseases of Economic Importance***

*Pancreas Disease (PD).* Pancreas disease (PD) is an important disease of farmed Atlantic salmon in seawater, particularly in Scotland and Ireland. It is characterized by a sudden and complete necrosis of the exocrine pancreas. Behavioural signs are loss of appetite and fish resting in corners of cages. Internally there may be petechial haemorrhaging in the caecal area but diagnosis requires histological examination of the pancreas. The disease can recur at any time in seawater, but once established on a site, outbreaks typically occur in July-August following transfer to the sea. Given good husbandry conditions, fish recover in several weeks. However, recovery is variable in two-sea-winter fish and the disease may progress into a wasting of body musculature and loss of weight. Such fish are culled and the economic loss may be considerable. Following an outbreak in post-smolts, populations are reputed to develop a strong immunity to further infection.

The disease can be transmitted experimentally (Raynard and Houghton 1993) and is presumed to be of viral aetiology. Experimental evidence indicates that pancreatic necrosis occurs 7-10 days after infection by injection and is dependent upon the fish feeding. If fish are not fed during this period no pathology develops but development of immunity is unaffected. This has led to a control practice whereby at the first signs of PD, the fish are taken off feed for several days. Some farmers report reduced severity of PD by this practice but a potential hazard is that if feed is withdrawn before most of the fish have been infected, and withdrawal of feed prevents the infection spreading through the population, then no immunity can develop and large numbers of the population are still at risk to subsequent exposure.

Recently Nelson et al. (1995) have isolated a toga-like virus from the pancreas of disease-affected Atlantic salmon. When inoculated into Atlantic salmon post-smolts it caused pathological changes in the pancreas indistinguishable from those observed in natural outbreaks of PD. It is proposed that the virus be named salmon pancreas disease virus (SPDV).

*Infectious Salmon Anaemia (ISA).* Infectious salmon anaemia (ISA) is a devastating disease of farmed seawater Atlantic salmon in Norway and has hitherto not been reported elsewhere. Typical signs are severe anaemia, pale gills, ascites, enlarged spleen and haemorrhagic necrosis of the liver (Thorud and Djupvik 1988). Mortalities vary between 15 and 100% with most outbreaks occurring in May and June but cases occur throughout the year. Transmission of the disease can be effected by injection of bacteria-free filtrates of tissue homogenates and plasma from infected fish. Virus-like particles have been observed by electron microscopy in blood cells and plasma filtrates, and they have been purified from these cells (Thorud et al. 1990; Christie et al. 1993). The virus causing ISA has recently been isolated in cell culture from Atlantic salmon kidney tissue (Dannevig et al. 1995). Electron microscopy studies indicate the agent is a budding, non-cytolytic RNA virus.

Fish that recover from ISA are less susceptible to reinfection and their sera can neutralize the ISA agent. This result gives promise that development of a recombinant sub-unit vaccine may be feasible if viral nucleic acid can be isolated from diseased tissues. As infection can be readily transmitted through the water, especially with blood, current control measures concentrate on high standards of hygiene and the containment and disinfection of blood and other waste from slaughter units to prevent spread of infectious material. Recent work suggests that salmon lice may be an important vector for ISA (Nylund et al. 1994*b*). Brown trout may represent natural reservoirs of the virus (Nylund et al. 1994*a*).

## BACTERIAL DISEASES OF SALMONIDS

### *Biology and Classification of the Main Groups Significant to Salmonid Culture*

Bacterial diseases are the most economically important diseases of salmonids and the need for their control in salmonid farming has been the main stimulus in developing good husbandry, chemotherapy and vaccination programs in aquaculture. Indeed, the susceptibility of certain salmonid species to certain bacterial pathogens has sometimes shaped salmonid culture. For example, the introduction of Atlantic salmon culture to the Pacific coast of North America occurred, in part, because of the greater resistance of Atlantic salmon to Bacterial Kidney Disease (BKD) compared with the indigenous Pacific salmon species which have suffered immense losses from this disease. The introduction of *Aeromonas salmonicida*, the causative agent of furunculosis, in the U.K. during the early 1900s and the consequent epidemic in wild Atlantic salmon, led to the first legislation to control the spread of a fish disease. While some bacterial diseases of salmonids have appeared only recently in certain parts of the world (e.g., *Yersinia ruckeri* has spread throughout Europe during the 1980s, as has furunculosis in Norway), most of the important bacterial diseases of salmonids can be regarded as having a wide distribution.

The bacterial diseases of salmonids, their causative agents and principal disease signs are shown in Table 2. Some of these agents are highly adapted pathogens and do not survive well outside their hosts, these are referred to as obligate pathogens (*see* Table 2). Others may be present in the natural flora of the aquatic environment; these are termed opportunistic pathogens. While the obligate pathogens can often reside in or on the fish producing a "carrier" or "latent" stage of infection where no disease signs are evident, actual outbreaks of bacterial diseases, whether caused by obligate or opportunistic agents, are usually associated with the fish being stressed. Hence, stress management (*see* below) is important in avoiding outbreaks of disease. Moreover, unless the stressful influences precipitating the outbreak are ameliorated, it is unlikely that chemotherapy or vaccination will be very effective in cure or prevention.

*Furunculosis.*

*1) Modes of expression in fish.* There are two forms of furunculosis in salmonids caused by two different subspecies of *Aeromonas salmonicida* referred to as typical or atypical strains. The most important economically is caused by the typical strain *Aeromonas salmonicida* subspecies *salmonicida*. The atypical strain, *A. salmonicida* subspecies *achromogenes*, is usually less virulent and causes economic loss in more restricted geographic regions. In Iceland it is regarded as a very important disease in Atlantic salmon farms but elsewhere it is a minor problem. The typical form of furunculosis is one of the most devastating diseases of cultured Atlantic salmon and while all species of salmonids are susceptible, the disease is less of a problem for culture of Pacific salmon and rainbow trout, which may carry the agent but are relatively resistant to disease outbreaks.

Atlantic salmon, both in fresh water and seawater are at risk. Essentially the disease has two forms, acute and chronic. In the former, there are few external signs other than darkening, anorexia, lethargy and a reddening of the fin bases. Internally there may be a widespread haemorrhaging to a greater or lesser degree. Death is caused by a septicaemic condition with the bacterium being found in all organs, and probably results from bacterial toxins inhibiting the function of vital organs and producing circulatory failure. Chronic disease is more often found in older fish and is characterized by acute haemorrhages, enlargement of the spleen, and liquefaction of the kidney. Furuncles, which give the disease its name, may or may not develop. They are caused by growth of the bacterium in the body muscle. The toxins released by the organism cause liquefaction of the tissue and an intense inflammatory reaction causing swelling. This lesion often ulcerates with release of the blood-stained liquefied tissue. This exudate

**TABLE 2**

Principal bacterial diseases in salmonids.

| Disease | Causative agent | Signs | Main host and geographic range |
|---|---|---|---|
| Furunculosis | *Aeromonas salmonicida* | Internal haemorrhaging, vasodilation, liquefied kidney, furuncles (except Chile) | All salmonids especially *Salmo* spp. FW and SW. Worldwide |
| Vibriosis | *Vibrio anguillarum/ ordalii* | Skin ulceration and haemorrhaging, pale gills. Petechial haemorrhage in visceral organs, swollen spleen, liquefied kidney | All salmonids in SW. Worldwide |
| Coldwater vibriosis | *Vibrio salmonicida* | Pale gills, petechial haemorrhages in ventral surfaces, swollen inflamed vent. Extensive haemorrhages internally, pale liver | Atlantic salmon, SW. Norway |
| Enteric redmouth disease (ERM) | *Yersinia ruckeri* | Reddening and ulceration of mouth region. Internally haemorrhaging and swelling of kidney and spleen | Rainbow trout, FW. Worldwide |
| Bacterial kidney disease (BKD) | *Renibacterium salmoninarum* | Darkening, reddening fin bases, exophthalmia. Swollen kidney with whitish patches. Pseudomembrane over visceral organs | All salmonids especially *Oncorhynchus* spp, FW and SW. Worldwide |

TABLE 2 cont'd.

| Disease | Causative agent | Signs | Main host and geographic range |
|---|---|---|---|
| Columnaris disease | *Flexibacter columnaris* | Whitish mucoid patches on skin and and gills overlying necrotic tissue | Rainbow trout, FW. Worldwide |
| Fin and tail rot | *Flexibacter columnaris* | Erosion of fins and tail | Salmonids, FW. Worldwide |
| Saddleback | *Flexibacter columnaris* | Grey patch around base of dorsal fin | Salmonids, FW. Worldwide |
| Salt water columnaris disease | *Flexibacter maritimus* | Erosion of fins, gill hyperplasia | Salmonids, SW. Tasmania |
| Bacterial gill disease | *Flavobacter branchiophila* | Swelling and fusion of gill filaments, excess mucus production by gills | Salmonids, FW. Worldwide |
| Cold water disease | *Flexibacter psychrophilus* | Severe erosion of tail fin and peduncle | Salmonids, especially coho salmon, rainbow trout fry, FW. Worldwide |
| Peduncle disease | | Severe erosion of tail fin and peduncle | |
| Rainbow trout fry anaemia | | Pale gills, anaemia | |
| Salmonid rickettsial septicaemia | *Rickettsia* | Mottled liver, enlarged kidney, empty gut | Salmonids, SW. Chile, Ireland |
| Pseudokidney disease | *Carnobacterium* | Petechial haemorrhage, exophthalmia. Haemorrhage in visceral organs | Salmonids, Europe, N. America |

is considered to be the main source of transfer of infection as it is rich in bacteria. For a detailed description of this disease, see Munro and Hastings (1993).

*Vibriosis.*

*1) Modes of expression in fish.* This disease is caused mainly by *Vibrio anguillarum*, but *Vibrio ordalii* and other *Vibrio* species may sometimes be the cause (Hjeltnes and Roberts 1993). It is a disease of salmonids in seawater where the bacterium is part of the environmental flora and may be present as part of the gut microflora of the fish. Outbreaks of the disease are associated with severe stress, particularly if the surface of the fish is damaged. Disease occurs at fairly high temperatures, i.e., above 10°C. Acutely affected fish show dark swollen skin lesions which ulcerate releasing a haemorrhagic fluid. Internally the spleen may be enlarged and the kidney liquefied. Petechial haemorrhages may be found in visceral organs and the body muscle, and the gills appear pale. From histological examination it is evident there are significant changes in the blood (Lamas et al. 1991, 1994).

*Cold Water Vibriosis.*

*1) Modes of expression in fish.* This disease has rarely occurred outside Norway. One outbreak occurred in Scotland (Bruno et al. 1986) and a further one in New Brunswick, Canada. In Norway it has caused major losses in seawater Atlantic salmon farms since the 1970s (Hjeltnes and Roberts 1993). As the name of the disease implies, the causative agent, *Vibrio salmonicida*, is active at low temperatures (about 7°C). The disease is septicaemic and many bacteria can be found in the circulation. The pathologic consequences that occur depend on the stage of the disease process. Early clinical signs of disease include inappetence and loss of balance, whereas during the later acute phase of the disease, pale gills, reddening of the fin bases, inflamed and swollen vent, and extensive petechial haemorrhages in the ventral surfaces may be recorded. Internally, extensive haemorrhaging of visceral organs, ascites and pale liver may be found.

*Enteric Redmouth Disease (ERM).*

*1) Modes of expression in fish.* ERM is caused by *Yersinia ruckeri* and is mainly a disease of young rainbow trout. However, in recent years the disease has occurred in seawater salmon farms in Norway. Outbreaks of ERM occur at temperatures above 10°C when carrier populations are stressed. A characteristic feature, but not one which is always present, is reddening of the mouth region due to congestion of blood vessels, ulceration and haemorrhaging. Internally, petechial haemorrhages in visceral organs and swelling of the kidney and spleen are typical signs of a generalized systemic haemorrhagic bacteraemia. The intestines are flaccid and often contain a yellowish fluid (Stevensen et al. 1993).

*Bacterial Kidney Disease (BKD).*

*1) Modes of expression in fish.* BKD has recently been reviewed by Evelyn (1993), Evenden et al. (1993) and Fryer and Lannan (1993). The disease is caused by *Renibacterium salmoninarum* which, unlike the other major bacterial pathogens of salmonids, is a Gram-positive organism. It is a very slow growing organism and for this reason presumptive diagnosis of BKD by standard bacteriological culture techniques may take several weeks. The bacterium is an intracellular pathogen being able to survive within the fish's macrophages (Bandin et al. 1993). However, it does not exclusively grow inside the host cells, and in later stages of the disease, extremely large numbers of bacterial cells may be present throughout the extensively necrotised kidneys. At this stage the bacteria are easily observed microscopically in Gram-stained smears. All salmonid species are considered to be susceptible but the Pacific salmon, especially coho and chinook, are the most seriously affected. BKD is a chronic disease condition and persists as a carrier state in infected populations when it is very difficult to identify. It affects both freshwater- and marine-cultured salmonids, although infection is believed to derive from the freshwater environment.

Clinical outbreaks tend to be seasonal as they are related to either declining or increasing temperature (Bruno 1986*a*). Transmission can occur horizontally or vertically as the bacterium can survive within ova. As such ova cannot be disinfected, it is very important that BKD-free brood stock are maintained. As BKD is a chronic condition low-level mortalities can eventually result in considerable loss of market-size fish. In farmed rainbow trout the disease is most prevalent in spring with mortalities peaking in early summer. Affected fish are usually dark in colour and sometimes exophthalmia and slight haemorrhaging at the bases of the pectoral fins occur. Internally, the swollen kidney may contain whitish nodules of granulomatous tissue in response to the bacterium. A greyish pseudomembrane may also be seen covering certain visceral organs, particularly the spleen (Bruno 1986*b*).

*Cytophaga-like Bacteria (CLB).*

*1) Modes of expression in fish.* This term has come to replace the term "myxobacteria" and includes the genera *Cytophaga*, *Flexibacter* and *Flavobacterium*, which have been associated with a variety of skin, fin and gill diseases of salmonids, particularly in hatcheries. Such diseases are almost invariably associated with poor water quality. This group of bacteria are Gram-negative rods or filaments which have a gliding motility and form yellow, orange or red colonies when cultured.

Columnaris disease caused by *Flexibacter columnaris* is a serious skin and gill disease in salmonid hatcheries. Signs are whitish mucoid patches consisting of swarms of the bacterium, surrounded by a reddish haemorrhagic zone and overlying necrosis of the epidermis. "Saddleback" disease, so-called because of a grey discoloration of the skin around the base of the dorsal fin, is also associated with *Flexibacter columnaris*. This may progress to become ulcerative and haemorrhagic. Outbreaks of columnaris disease usually occur at fairly high temperatures (above 15°C).

Bacterial gill disease is a common problem in salmonid hatcheries. It is associated with *F. columnaris* but perhaps more significant is *Flavobacter branchiophila* (Turnbull 1993). Signs are principally related to: (1) the anoxic condition resulting from increased mucus production and swelling of the primary gill lamellae, and (2) fusion of the secondary gill lamellae in response to the bacterial colonisation. Thus, the fish are lethargic, anorexic and remain near the water surface or inlet flaring their opercula and coughing. Several types of CLB have been isolated from eroded fins and tails of salmonids but some workers consider the bacteria are secondary invaders following trauma, e.g., fin-bites. The disease may occur over a wide temperature range.

Cold-water disease or peduncle disease is a freshwater disease occurring at temperatures of 4-12°C with losses decreasing sharply at higher temperatures. The agent is *Cytophaga psychrophila* (= "*Flexibacter psychrophilus*") (Dalsgaard 1993), which colonizes the epidermis resulting in an epidermal hyperplasia with increased mucus production and a greyish thickening appearance to the lesion. Affected fish (1-2 g) may show increased pigmentation, loss of balance, abdominal swelling, exophthalmia and pale gills, with larger fish (up to 60 g) also exhibiting regular subcutaneous lesions at one or more sites on the body surface (Bruno 1992*a*). Underlying necrosis ensues and in the case of peduncle disease, the entire tail fin may be completely eroded. This same bacterium has recently been identified as the cause of Rainbow Trout Fry Syndrome (RTFS), which is a systemic form of the disease resulting in severe anaemia and heavy losses in rainbow trout hatcheries (Santos et al. 1992).

### *Minor Bacterial Diseases of Salmonids*

*Aeromonas hydrophila*. *Aeromonas hydrophila* is the causative agent of motile aeromonad septicaemia. The bacterium is normally present in the aquatic environment, especially in warm, organic-rich fresh water. As such environments are not suitable for most forms of salmonid culture, this disease is mainly restricted to rainbow trout culture in warm countries. There is some debate as to whether *A. hydrophila* is a primary pathogen of fish as infection is usually associated with severe stress, physical trauma or following infection by another pathogen.

*Pseudomonas fluorescens*. *Pseudomonas fluorescens* is an opportunistic pathogen and may cause a haemorrhagic bacteraemia or superficial ulcer formation in salmonids associated with elevated temperatures and poor husbandry conditions. The bacterium is widely distributed in soil and water.

*Pseudomonas anguilliseptica*. *Pseudomonas anguilliseptica* has been isolated from farmed Atlantic salmon, sea (brown) trout and rainbow trout in Finland. Gross signs of infection include petechial haemorrhages in the skin, peritoneum and liver. Antibiotic therapy with oxytetracycline has not been successful (Wiklund and Bylund 1990).

*Streptococcus spp*. Streptococcal septicaemia (*Streptococcus* spp.) has been reported in several farmed fish species, but in salmonids it has only been reported in rainbow trout in Japan, South Africa, Australia and Italy when water temperatures exceed 20°C. Affected fish develop an acute septicaemia often with high mortalities.

*Mycobacteriosis and Nocardiosis — Modes of Expression in Fish.* *Mycobacterium chelonei* has been reported to cause disease in Pacific salmon hatcheries in North America and Australia. *Nocardia asteroides* has caused disease in rainbow trout and brook trout hatcheries in North America but these outbreaks appear to be very rare.

*Lactobacillus piscicola.* *Lactobacillus piscicola* has been associated with a so-called pseudokidney disease in mature rainbow trout for many years. It appears to be a stress-related disease with low mortality and its main significance is its similarity to *Renibacterium salmoninarum* in Gram-stained kidney smears. However, it is readily distinguishable from the latter by serological and biochemical tests (Austin and van Poucke 1993). On the basis of biochemical, physiological and chemical criteria, *Lactobacillus* has been placed into a new genus, *Carnobacterium* (Collins et al. 1987).

*Epitheliocystis and Salmonid Rickettsial Septicaemia.* Two unusual obligate intracellular micro-organisms have been identified as causing disease in salmonids. Epitheliocystis growing within gill epithelial cells, which become swollen, appears as small whitish cysts on the gills. Damage is slight. On the other hand, rickettsial septicaemia, caused by *Piscirickettsia salmonis*, has been associated with severe mortalities in Atlantic, coho and chinook salmon and rainbow trout from seawater sites in Chile (Cvitanich et al. 1991; Lannan and Fryer 1993). Recently, an outbreak of piscirickettiosis has occurred in Chile among rainbow trout reared in fresh water (Bravo 1994). Rodger and Drinan (1993) have also reported on a similar rickettsia-like organism in Atlantic salmon post-smolts from the west coast of Ireland.

### *Modes of Prevention and Control*

*Exclusion.* Most of the major bacterial diseases of salmonids are virtually endemic worldwide and only farms with protected water supplies, e.g., well water, are not at risk of contracting these diseases. The rickettsial septicaemia in Chile and cold water vibriosis (*Vibrio salmonicida*) in Norway are the only examples of serious diseases of salmonids having a limited geographic distribution (Table 2).

*Husbandry.* Many bacterial diseases have little clinical effect in healthy stocks even if carriers are present. Disease outbreaks are mainly associated with stressful events such as grading and transportation, but poor husbandry and environmental conditions are major factors. The latter should therefore be avoided or alleviated by improving water quality, reducing population density and paying special attention to hygiene, e.g., removing dead and moribund fish. Most of the obligate pathogens do not survive for long periods outside their hosts; therefore various practices on farms such as tank, raceway and pond clearing and disinfection will reduce infection. A special case in cage culture involves fallowing sea-lochs for a few weeks after harvest; this has greatly reduced or eliminated outbreaks of sea-lice infestations and furunculosis in Scottish salmon farms.

*Chemotherapy.* Antibiotics and other chemicals have been invaluable in controlling many bacterial diseases in aquaculture but problems have commonly arisen with development of antibiotic resistance in the bacteria. As an example, oxolinic acid was widely used in Scotland to control furunculosis outbreaks in salmon farms. However, the first cases of the appearance of strains of *A. salmonicida* resistant to oxolinic acid were reported in 1987, and by 1991 almost half of the isolations made from outbreaks in 1987 were resistant (Inglis et al. 1991). Resistance to oxytetracycline and trimethophrim is also prevalent (Inglis et al. 1993*a*). Further concerns on the use of antibiotics in aquaculture relate to environmental effects (*see* Chapter 16). In addition, restrictions are enforced concerning withdrawal times to ensure no residues are present in carcasses when fish are harvested.

*Vaccination.* As a result of the problems with chemotherapy, a great deal of research now concerns developing vaccines. However, for economic reasons, the use of vaccines in aquaculture dictates that the cost of individual doses be extremely low. Hence, the method of producing vaccines against fish diseases has to be simple and inexpensive. Thus, only whole cultures of bacteria, chemically inactivated and without requiring further purification stages, have been used as vaccines. As such, highly effective vaccines are in commercial use for vibriosis, cold-water vibriosis and ERM. With the use of oil adjuvants and growth of the bacterium under iron-restricted conditions, efficacious vaccines against furunculosis are now available.

## FUNGAL DISEASES OF SALMONIDS

### *Biology and Classification of the Major Groups Significant to Salmonid Culture*

A range of taxonomically diverse fungal species are responsible for serious and economically important diseases in farmed and wild fish; these act both as secondary as well as primary agents of disease. Mycoses frequently occur as a consequence of stress and sexual maturity, as well as directly affecting eggs and fry. In most fungal outbreaks there is often one or possibly several predisposing factors that will affect the fish or the fungus. These include pollution, low water levels, overcrowding, mechanical trauma such as handling, change in hormonal status, the failure to remove moribund, or dead fish or ova, as well as infection by other bacterial, viral or parasitic agents. Primary agents of disease usually infect and multiply within the host tissues, having gained entry either through surface abrasions or from infections originating in the intestinal tract, whereas secondary agents generally infect the surface epithelium, often spreading over large areas (Miyazaki et al. 1977). A further, although arbitrary, division of such fungi may be related to the age of the group affected (e.g., eggs, brood stock) and whether outbreaks occur in fresh or salt water.

The most widely distributed mycotic infections of cultured salmonids are those caused by the freshwater moulds, Oomycetes, and follow bacterial infections in terms of their economic importance in aquaculture. They are separated from other classes of fungi by the fact that they have some cellulose in their cell walls and produce motile spores with two flagella. The motile zoospores are produced in structures called zoosporangia and in general represent both the primary means of asexual reproduction and dispersal. Within the class Oomycetes there are four orders but the representatives of the family Saprolegniaceae cause the majority of the reported infections of living fish, with the genus *Saprolegnia* being the most significant. There are two principal species involved in disease outbreaks, *S. parasitica* and *S. diclina*, although this taxonomically difficult group is often referred to as the *Saprolegnia* complex (Neish and Hughes 1980; Willoughby 1985). Other genera involved as salmonid parasites within the Oomycetes include: *Achlya*, *Aphanomyces*, *Dictyuchus*, *Leptolegnia*, *Pythiopsis*, *Thraustotheca* and *Pythium*, although clear experimental evidence of pathogenicity in fish has not been reported in all cases and others have only been associated with infrequent outbreaks.

The taxonomy of the genera *Ichthyophonus* and *Exophiala* is not well researched. The former genus is currently placed in the Entomophthorales group. The genus *Branchiomyces* has been placed tentatively in the Saprolegniaceae. *Phoma herbarum* is a widespread plant saprophytic fungus belonging to the Coelomycetes. The classification of fungal diseases affecting salmonids is summarized in Table 3.

*Saprolegnia.*

*1) Modes of expression in fish.* A cottony-white mycelium on the integument of the fish and the surface of eggs makes preliminary identification of *Saprolegnia* spp. relatively easy. This fungus grows by producing long filamentous strands, called hyphae. Early epithelial lesions appear as circular foci on the head and dorsal areas of the body surface, which progressively spread laterally to produce large irregular patches; these may develop into acute dermal ulcers with local haemorrhaging, epidermal sloughing and subdermal lateral spreading. The rate at which these lesions develop is determined by such factors as the water temperature and condition of the fish. Diseased fish become increasingly lethargic with a loss of equilibrium shortly before death. The gills, olfactory pits and eyes may also become infected. Precocious male parr are particularly prone to saprolegniasis and in wild salmon and trout, *Saprolegnia* infections are common before, during and after spawning. Dead eggs readily become infected and they should be removed quickly thus preventing the hyphae spreading to adjacent healthy eggs.

Histologically the branched, non-septate hyphae of varying diameter colonise and penetrate the outer and then the deeper skin layers and may sometimes even penetrate the superficial musculature. Under certain conditions *Saprolegnia* spp. can act as a

**TABLE 3**

Classification of fungal diseases affecting salmonids.

| Genus | Group | Occurrence | Distribution |
|---|---|---|---|
| *Achyla* spp. | Saprolegniaceae | Fresh water | U.S., Japan |
| *Aphanomyces* | Saprolegniaceae | Fresh water | U.S. |
| *Branchiomyces* | Saprolegniaceae | Fresh water | U.S., Japan, E. Europe |
| *Saprolegnia* spp. | Saprolegniaceae | Fresh water | Worldwide |
| *Thraustotheca* | Saprolegniaceae | Fresh water | |
| *Pythium* sp. | Leptomitaceae | Fresh water | U.S. |
| *Exophiala salmonis* | Moniliales? | Seawater | Canada, Europe |
| *Ochroconis tshawytschae* | Hyphomycetes | Fresh water | U.S., Canada |
| *Ichthyophonus hoferi* | Entomophthorales | Mainly seawater | Worldwide |
| *Phoma herbarum* | Sphaeropsidales | Fresh water | Northwest U.S. |
| *Paecilomyces farinosus* | Deuteromycetes | Fresh water | U.K. |
| *Phialophora* sp. | Deuteromycetes | Fresh water | U.K. |

primary pathogen as reported in amago salmon (*Oncorhynchus rhodurus*) fry (Hatai and Egusa 1977).

Most of these outbreaks can be taxonomically identified as *S. diclina*, although this species is often grouped with *S. parasitica* and considered as the *S. diclina-parasitica* complex. Among a commercially reared population of Atlantic salmon, first-feeding fry mortalities have occurred as a direct result of infection by *S. diclina* (Bruno and Stamps 1987). A variable mortality over a six-week period resulted in a loss in this population of 27%. Hyphae and other debris blocked the pharynx and the hyphae had grown outward over the gill lamellae preventing feeding and normal respiratory movements. Such infections can result in necrosis of the gill lamellae and distension of the opercular cavity. *S. diclina* possesses distinctive bifurcate hooks on the secondary zoospore cysts that may assist with attachment to the host skin (Pickering et al. 1979). Isolates of *S. diclina* readily encyst, germinate and grow in dilute nutrients and have enhanced growth in water "conditioned" by fish such as hatchery effluent (Noga 1993). *Saprolegnia* spp., grow on a variety of media. The hyphae are weakly PAS-positive (Wolke 1975).

*2) Methods of prevention and control.* Many chemicals have been tested for their effectiveness in controlling *Saprolegnia* infections, including malachite green, copper sulphate, crystal violet, sodium chloride and potassium dichromate. The use of zinc-free malachite green remains the most popular treatment as it is inexpensive and effective for the treatment of fungal skin infections. It has, however, been suggested as a possible teratogen and is no longer approved for use in all countries, and therefore, where used, care should be taken in handling the dry powder. A prophylactic treatment during egg incubation is recommended. When treating a large number of fish a bath or flush method is the most economic. For small numbers of fish a dip treatment of 30 seconds is advised. Patches of fungus on brood fish can be treated by applying the malachite green directly to the affected area through a syringe while the fish remains in the water. This method results in the now green-coloured fungus sloughing from the fish within 24-48 h.

All such bath or flush treatments should be tested on a small number of animals before large numbers are considered for treatment. The maintenance of fish under good husbandry conditions, i.e., avoidance of overcrowding, traumatic injuries, correct feeding and good water quality, will undoubtedly help to prevent *Saprolegnia* outbreaks.

*Exophiala.*

*1) Modes of expression in fish. Exophiala salmonis* is an internal mycotic disease, typical of a fungal mycetoma affecting salmonids reared in the marine environment, and was first described by Carmichael (1966). In general, infrequent outbreaks occur that affect a low number of fish in a population. The life cycle and predisposing conditions for infection are poorly understood although there is some evidence that infection may start from contaminated food.

Affected fish exhibit erratic swimming movements, which may be followed by whirling behaviour, exophthalmia and cranial cutaneous ulcers (Richards et al. 1978). Infected fish may continue to feed normally. A massive distension of the abdomen generally occurs due to the gross enlargement of the posterior kidney, which contains raised, greyish-white nodules, up to 3.5 cm in diameter containing variable quantities of fungal hyphae. The host attempts to wall off the fungus with the development of a marked, systemic granulomatous response involving many multinucleate giant cells and epithelioid cells followed by fibrosis and atrophy. Healing lesions are often fibrous in nature. Hyphae may enter the kidney tubules and blood vessels as well as other organs such as the heart, liver and spleen. The muscle may be affected in heavy infections in which case the flesh becomes discoloured.

*Ichthyophonus.*

*1) Modes of expression in fish.* Infection by the obligate fungal pathogen *Ichthyophonus hoferi* has been reported from a few fish species in fresh water, but principally infection occurs in stocks in the marine environment. This fungus is found in coastal and estuarine areas worldwide where it causes a chronic, internal systemic infection. This fungal pathogen has been the cause of death of large numbers of herring (Clupeidae). While it is a less serious disease for salmonids (the condition has not been recorded in marine salmon farms) these fish must be considered vulnerable. Infections in trout farms are believed to have come from feeding with unpasteurized fish products infected with the fungus.

External symptomatic features of *I. hoferi* infection in fish are not specific; spiralling, whirling and spinal curvature have been reported. Internally a whitish speckling and the presence of raised nodules on the muscle and internal organs resulting from an inflammatory response to the fungus are common features. This ultimately progresses to the formation of a granuloma (McVicar and McLay 1985). Within two to four weeks of infection the infective stages of *Ichthyophonus* become lodged in tissues initiating a lymphocytic response. Larger mononuclear cells aggregate around these spores with the formation of an organized encapsulating layer. Multinucleate giant cells and peripheral fibrosis of the granuloma are characteristic of well established infections. In some cases extensive invasion of the muscle has rendered carcasses unsuitable for sale. The gross appearance of the lesions can lead to erroneous association of *Ichthyophonus* with other granulomatous conditions.

*2) Methods of prevention and control.* Where epizootics of *Ichthyophonus* have occurred in freshwater sites rearing rainbow trout, the inclusion of unpasteurized marine fish as part of the diet has been identified as the source of infection. With the absence of any chemotherapeutic agents, avoidance of this disease by not using this dietary source is the only rational form of control.

*Phialophora.*

*1) Modes of expression in fish. Phialophora* spp. causes a systemic fungal infection in Atlantic salmon parr and is confined to the internal organs. It involves a new species of *Phialophora* which was first recorded in late January 1982 at water temperatures of 5-6°C (Ellis et al. 1983). Diseased fish exhibited haemorrhages at the base of the fins, were emaciated, and the abdominal area was distinctly swollen. Mortalities continued for three months.

Internally, firm whitish-pink fungal masses adhere to most of the internal organs, although principally to the posterior area of the kidney and intestine. Internally the liver and spleen may be pale with severe inflammation of the lower intestine. In culture this fungus is slow growing and the appearance of the colonies suggested that it is a member of the hyphomycetes, the so-called "black yeasts".

*2) Methods of prevention and control.* Since these "yeasts" tend to be opportunistic and present in many environments, infection by this pathogen may come from food that has become contaminated through bad storage; hence in most cases, this pathogen can be avoided.

*Branchiomyces.*

*1) Modes of expression in fish.* Two species of the genus *Branchiomyces* are parasitic on the gill tissues of salmonids where they cause a typical "gill rot" at temperatures above 10°C. The course of the disease is usually rapid and is enhanced by high water temperatures and overcrowding. The most frequently affected fish are carp (*Cyprinus carpio*) although tench (*Tinca tinca*), rainbow trout and pike have also been infected. Both brown and rainbow trout are considered susceptible but there has been no outbreaks of this disease reported in recent years. The invasive fungal hyphae obstruct the circulation of the blood through the gills. Diseased areas may slough off and the host tissue may then become the focus of infection for other fungi such as *Saprolegnia*. Outbreaks appear to be patchy although mortalities up to 50% have been recorded.

*2) Methods of prevention and control.* An increased water flow and good hygiene are recommended for affected fish with attention paid to strict hygiene including the removal of dead fish, increasing water supply and avoidance of over-feeding, especially at high water temperatures. Prevention of branchiomycosis can be accomplished with management techniques that provide good water quality, but holding fish in malachite green solution for 24 h is also reported as being successful.

*Paecilomyces farinosus.*

*1) Modes of expression in fish.* On several occasions a fungal infection involving the swim bladder of farmed Atlantic salmon parr has been encountered (Bruno 1989). Grossly, the abdomen of affected fish is enlarged and the vent area red and swollen. A large fungal mass, located in the swim bladder, appears to be confined to this organ. The causative agent has been identified as *Paecilomyces farinosus*, which is a reported pathogen of insects and reptiles. One source of infection may be from ingested insects. Alternatively, as most outbreaks involved fish reared in tanks with covers, it is suspected that insufficient air exchange under these circumstances could result in an accumulation of spore numbers.

*2) Methods of prevention and control.* In all cases examined mortalities have been restricted to a low number of fish, hence, therapy or control measures have not been researched.

### *Minor Fungal Diseases*

*Achlya.* Around 13 species of *Achlya* have been reported (summarized by Noga 1993), which appear to produce a variety of clinical symptoms in fish (e.g., brook trout) infected in fresh water. In some cases symptoms appear more severe when diagnosed as a secondary pathogen.

*Ochroconis.* Since the first report of *Ochroconis tshawytschae* (syn. *Scolecobasidium*) affecting chinook salmon (Doty and Slater 1946), all additional papers have considered *O. humicola* or an additional unnamed species to be the cause of visceral mycosis in rainbow trout, coho or masu (*Oncorhynchus masou*) salmon. The disease is characterized by enlarged kidney, reddening of the anal area, abdominal swelling and enlargement of the kidney by brown or dark mycelial aggregates (Ajello et al. 1977; Hatai and Kubota 1989).

*Phoma. Phoma herbarum* was first reported as a pathogen of rainbow trout and coho and chinook salmon by Ross et al. (1975). Affected fry exhibit swollen haemorrhagic vents and fins and may swim on their sides or in a vertical upside-down position. Internally, mycelia may be observed growing within the swim bladder. A granulomatous condition within the internal organs, particularly the kidney has also been noted.

*1) Methods of Prevention and Control.* No effective treatment or control measure has been reported for *Phoma* or *Ochroconis*, although infection by the latter is attributed to contaminated water sources and, hence, it is recommended that the water is kept clean and free of organic matter.

## PROTOZOAN PARASITES OF SALMONIDS

### *Biology and Classification of the Major Groups Significant to Salmonid Culture*

The protozoa are a diverse group of unicellular eukaryotic organisms that, in most cases, possess a monogenetic life cycle. Many protozoans are described from salmonids worldwide and only a limited description can be given here. The most important protozoan parasites of salmonids include: *Ceratomyxa shasta*, *Ichthyobodo necatrix*, *Myxobolus cerebralis*, *Kudoa thyrsites*, *Hexamita salmonis* and the PKX organism. Such parasites can multiply on the fish integument and in the viscera and skeletal system, and cause serious outbreaks of disease in cultured fish.

Protozoa parasitic on fish are mostly classified into six phyla: Mastigophora, Sarcodina, Microspora, Apicomplexa, Ciliata and Cnidosporidia. Both *Ceratomyxa* and *Myxobolus* are placed in the phylum Cnidosporidia, class Myxosporea, which have a well developed trophozoite stage generally with two, but up to six polar capsules. Trout and salmon whirling disease (WD), a persistent and economically important condition in hatcheries, is caused by a myxosporean protozoan *Myxobolus cerebralis* (Myxozoa: Myxosporea), previously known as *Myxosoma cerebralis* (Halliday 1976).

The bodonid flagellate *Ichthyobodo necatrix* [*Costia necator*] belongs to the phylum Mastigophora, class Zoomastigophora. Other related flagellates include *Hexamita* and *Cryptobia*. Proliferative kidney disease (PKD) is caused by a poorly understood myxosporean (PKX) which at the present time is thought to be a member of the family Sphaerosporidae, genus *Sphaerospora*. Within the phylum Ciliata, class Holotricha is the ciliate *Ichthyophthirius multifiliis*, which is distinguished by the uniform, heavy ciliature on the trophozoites. *Trichodina* is placed in the class Peritricha. The haplosporidian *Dermocystidium* is poorly understood taxonomically and it is possible several groups are represented (Lom and Dyková 1992).

Under normal conditions protozoan infections do not generally cause substantial losses or pathology of affected fish. However conditions such as overcrowding or poor water quality and other changes in environmental conditions may allow the parasite to rapidly increase in numbers. This may result in weight loss in the host, blindness, osmotic distress, susceptibility to predation and an increased chance of invasion by secondary pathogens. The management or prevention of such outbreaks requires a thorough understanding of the parasites' life cycle with correct identification of each parasite. The classification of some important protozoans affecting salmonids is shown in Table 4.

*Proliferative Kidney Disease.*

*1) Modes of expression in fish.* Proliferative kidney disease (PKD) is an economically important protozoan disease of cultured salmonids in fresh water, particularly rainbow trout in Europe and North America (Hedrick et al. 1993). Atlantic salmon and brook trout appear to be relatively resistant. The parasite is restricted to the family Salmonidae, with the exception of pike (Seagrave et al. 1981). Externally there are no distinguishing features in light infections, although during severe outbreaks affected fish show bilateral exophthalmos, darkened body, pale gills and abdominal distension. Internally a severe anaemia, presence of abdominal fluid, splenomegaly, and renal hypertrophy often with a mottled grey appearance due to a diffuse granulomatous nephritis, are noted. PKD generally occurs during the summer months. Compromised renal function from a degeneration of nephrons, and anaemia render affected fish susceptible to secondary pathogens, and overcrowding and poor water quality all contribute directly to mortalities.

**TABLE 4**

Classification of some of the important protozoans affecting salmonids.

| **Protozoan** | **Family** | **Host and environment** | **Location** | **Geographic range** |
|---|---|---|---|---|
| *Ichthyobodo necatrix* | Bodonidae | All freshwater fish, some occurrences in marine environment | Gills and body surface | Cosmopolitan |
| *Cryptobia salmonsitica* | Bodonidae | Species found in both environments | Parasite of the blood | Serious problem in U.S. hatcheries |
| *Trypanoplasma salmositica* | Bodonidae | *O. nerka*, variability among stocks. *O. mykiss* in fresh water | Blood parasites causing progressive anaemia | Lethal parasite. Pacific coast of N. America |
| *Hexamita salmonis* | Hexamitidae | Mainly fresh water<br>*O. mykiss, S. trutta*<br>*S. salar* in marine environment | Occurs in intestine and gall bladder. Locomotory disorders. Anorexia | Norway, Japan |
| *Thecamoeba hoffmani* | Thecamocbidae | *O. kisutch, O. tshawytscha, O. mykiss* in fresh water | Gills of cultured fish causing respiratory distress | Severe mortalities in U.S. hatcheries |
| *Paramoeba pemaquidensis* | Paramoebidae | Salt water pens rearing *S. salar, O. kisutch* | Gills affected causing epithelial hyperplasia. Can be severe | Australia and N. America. Opportunistic pathogen |

**Table 4 con'td.**

| Protozoan | Family | Host and environment | Location | Geographic range |
|---|---|---|---|---|
| *Ceratomyxa shasta* | Ceratomyxidae | *O. kisutch, S. trutta, S. fontinalis,* in fresh water | All layers of intestinal wall, spleen, kidney. Significant losses in farmed and wild fish | N. America, west coast |
| *Myxidium minteri* | Myxidiidae | Common in salmonids including *O. kisutch, O. mykiss* and *O. tshawytscha* in seawater | Infects gall bladder and liver, causes renal degeneration | Pacific NW of America |
| *Myxidium truttae* | Myxidiidae | *S. salar* and *S. trutta* in fresh water | Infects gall bladder in fresh water causing suppurative liver necrosis | Europe |
| *Henneguya zschokkei* | Myxobiolidae | Fish affected as juveniles and carry protozoan into seawater | Musculature. Causes a "milky-fluid" in the flesh making fish unmarketable | N. America, Asian coast |
| *Myxobolus cerebralis* | Myxobolidae | *Salmo, Oncorhynchus, Salvelinus, Hucho* spp. | Cartilage, causing whirling, black tails, emaciation and death | Hatcheries in N. and S. America, Australia and New Zealand |
| *M. neurobius* | Myxobolidae | *S. trutta* | Central nervous system | |
| *M. articus* | Myxobolidae | *O. nerka* | Central nervous system | |
| *M. kisutchi* | Myxobolidae | *O. kisutch* | Central nervous system | |

**TABLE 4 con'td.**

| **Protozoan** | **Family** | **Host and environment** | **Location** | **Geographic range** |
|---|---|---|---|---|
| *Loma salmonae* | Glugeidae | *O. mykiss, O. masou, O. nerka* in fresh water | Affects gill lamellae with considerable mortalities | Widespread in N. America, Japan. Imported into France |
| *Enterocytozoon salmonis* | Enterocytozoonidae | *O. tshawytscha, O. mykiss* in seawater | Occurs in the nucleus of haematopoietic cells of urinary tubules. Acute anaemia mortalities | Pacific NW of the U.S., Canada and France |
| *Sphaerospora truttae* | Sphaerosporidae | *S. trutta* in fresh water | Occurs in renal tubules causing atrophy | Central Europe |
| PKX organism | Sphaerosporidae | *O. mykiss, O. kisutch* and *O. tshawytscha* in fresh water | Primarily in the kidney | Europe, N. America, Canada |
| *Chloromyxum truttae* | Chloromyxidae | *S. trutta, S. salar, O. mykiss* in fresh water | Gall bladder. Emaciation, yellow colour to fins, fatal | Russia |
| *Ichthyophthirius multifilliis* | Ichthyophthiriidae | Freshwater fish | Surface tissues subacute hyperplasia | Cosmopolitan |
| *Trichodina truttae* | Trichodinidae | Salmonids in fresh water | Skin surface | N. America, Russia |
| *Kudoa thyrsites* | Kudoidae | *S. salar* smolts. *O. gorbuscha* in seawater. *O. mykiss, O. nerka* in fresh water | Muscle fibres, swollen kidneys, anaemia and death | U.S., Canada, Ireland |

Diagnosis relies on the associated pathology and the microscopic detection of the extrasporogonic stages of PKX in stained kidney sections or smears. More recently this has been aided with the development of monoclonal antibody and lectin probes (Adams et al. 1992). These probes recognize developmental and sporogonic stages of PKX (Hedrick et al. 1993).

*2) Methods of prevention and control.* Although fish surviving an infection appear to be resistant to reinfection, the basis of acquired immunity is unknown, but likely to result from the strong humoral and cellular responses to infection. Periodic treatments with malachite green administered as short baths, or oral treatment with fumagillin have been shown to reduce the severity or onset of PKX infections, although avoidance of contact between the pathogen and the fish has been the most successful approach in reducing the effects of PKD in hatchery-reared salmonids.

*Ceratomyxa shasta.*

*1) Modes of expression in fish. Ceratomyxa shasta* is a myxoporidian parasite of various Pacific salmon species in fresh water, occurring principally in farmed fish along the northwest of the U.S. and British Columbia where it contributes significantly to hatchery as well as pre-spawning mortalities in both salmon and rainbow trout (Conrad and Decew 1966; Sanders et al. 1970). Disease is caused by the spores of the parasite infecting the gut and internal organs. Infected juvenile rainbow trout are typically dark, lethargic and become anorexic. Abdominal distension with ascites, exophthalmia, and a swollen, haemorrhagic vent are also common (Schäfer 1968). In adult salmon, swollen and haemorrhagic areas develop in the intestine, with perforation of the intestinal wall and consequently peritonitis. Nodular lesions may develop in the viscera. Diagnosis involves the microscopic demonstration of typical spores from the intestinal lumen, gall bladder or abscessed lesions within the body musculature. Susceptible fish may become infected by coming into contact with water containing the infective stage. At temperatures between 4-6°C progress of the disease is slow, whereas at 23°C mortalities occur within 12-13 days.

*2) Methods of prevention and control.* Considerable differences exist in susceptibility among salmonid species and within strains. Juvenile salmonids originating from waters where the infective stage of the parasite is endemic have been reported as more resistant than strains of fish living in areas free of the infective stage (Buchanan et al. 1983). Crosses made between resistant and susceptible strains of coho salmon are intermediate between the parental stocks (Hemmingsen et al. 1986). Hence the movement of salmonids from non-endemic areas into endemic areas is not likely to be successful as interbreeding may adversely affect the survival of resident resistant fish. The management of *C. shasta* in areas where it is endemic requires that infected water supplies should be treated by filtration followed by chlorine or ultraviolet (UV) radiation (Sanders et al. 1972). However, once fish become infected there is no known effective means of eliminating the disease.

*Ichthyobodo necatrix.*

*1) Modes of expression in fish.* The bodonid flagellate *Ichthyobodo necatrix* is a widespread ectoparasite of freshwater fish, which often becomes prevalent among cultured salmonid fry and fingerlings on the gills or the body surface. Several reports have identified marine forms of this protozoan both on non-salmonids and Atlantic salmon (Diamant 1987; Bruno 1992*b*). Extensive infestations of the skin and gills often result in an acute and erosive epithelial hyperplasia and exhaustion of the goblet cells followed by heavy mortalities (Ellis and Wootten 1978).

In fresh water, young fry are particularly susceptible and often appear grey in colour because of a mass of parasites and thickened skin epithelium. Presumably as a result of the irritation, fish exhibit "flashing" movements in the water.

The free-swimming stage of *I. necatrix* is ovoid to kidney-shaped with two pairs of flagella. Parasites attach by means of a plate that in fresh water possesses ridge-like projections along much of the length of the cytostome process (Joyon and Lom 1969). The attachment plate penetrates epithelial cells by which the parasite absorbs nutrients. Marine forms of *Ichthyobodo* do not possess ridges on the cytostome (Lamas and Bruno 1992) suggesting the marine forms are a different species.

*2) Methods of prevention and control.* Control of this parasite in fresh water can readily be achieved using formalin as a bath treatment. Treatment in cages is difficult and not always successful. Fingerling and yearling fish appear more resistant to infection and different species vary in resistance to infestation; however, this latter area has received little attention.

*Myxobolus cerebralis.*

*1) Modes of expression in fish.* Whirling disease (causative agent *Myxobolus cerebralis*) was first reported in 1893 from imported rainbow trout (Hoffmann 1990). At the present time around 29 countries have reported the occurrence of WD. In many countries the first records occurred during the late 1960s and are believed to result from shipping infected live and frozen fish and fish products prior to the emergence of certification procedures.

The fish become infected by the small amoeba-like trophozoite stage. The trophozoite grows and divides producing pansporoblasts, each of which will produce two spores which localise in the cartilage. *M. cerebralis* is almost always found in the skeletal parts of the fish and rarely in granulomas involving other parts of the skeleton. This feature helps to distinguish it from other myxosporean parasites that cause whirling of fish, but which are not found in the cartilage. The spores of *M. cerebralis* are ovoidal, with a piriform-shaped polar capsule at the anterior end. The two polar capsules are normally of equal size. Affected fish often exhibit frenzied, tail-chasing behaviour.

Clinical signs are seen in fry and include blackened trunk and tails, tail chasing and whirling. The latter behaviour is often more pronounced when the fish attempt to feed or when they are startled. Skeletal deformities are generally confined to the cranial areas, jaws and opercula. Due to the difficulty of conclusively identifying the trophozoite, confirmation of WD is made by identifying the spores microscopically, either from stained tissue sections (e.g., Mallory-Heidenhain, Giemsa), or from wet mounts of macerated cartilage. The development of the spores in the fish has been linked to their acquisition of acid-fastness when they have been referred to as "mature".

Fish become infected during their first year of life, often in the first few weeks of active feeding. From observations on experimentally infected fish, the trophozoite incubation period varies between 40 to 90 days. After several months the spores will be present in the cartilage of the head and vertebrae where they induce necrosis and lysis either directly or indirectly. As ossification proceeds, osteogenesis is disrupted resulting in permanent cranial and other deformities in the fish. Erosion of the cartilage surrounding the auditory organ is reported to contribute to the typical whirling behaviour. Infection of the cartilage of the spinal column causes pressure on the caudal nerves resulting in reduced control over the production and distribution of melanin pigments, and is observed in the fish as blackened tails. Although some fry die before any clinical signs of WD appear, in most cases the course of the disease is chronic and spores are likely to remain in infected fish for at least three years.

In the past, WD has only rarely been detected in wild fish, but its occurrence has increased in the western U.S. Among farm-reared salmonids, most species from the genera *Salmo*, *Oncorhynchus* and *Salvelinus* are susceptible. Brown trout are reported not to show any signs of disease unless infected when very young.

The life cycle involves a parasitic phase and a free-living phase. Ingested spores are thought to leave the spore capsule in the intestine and migrate as trophozoites through the intestinal wall to the cranial cartilage. It is generally assumed that the spores are the infective stage and are probably released after death of the host. Final development for reinfection appears to take place once the spores are released from the fish. This "ageing" process may take three months or more (Halliday 1973). Development of infectivity requires the participation of an aquatic tubificid oligochaete worm, *Tubifex tubifex*, as an intermediate host (Markiw and Wolf 1983) in which the ingested spores further develop through to the triactinomyxon form (Markiw 1986). Therefore two separate stages of sporogeny occur, one in each host. Reinfection in the salmonid host may occur through the skin and buccal cavity and initiates the *M. cerebralis* infection. In rainbow trout successful development of the parasite only occurs in sac fry older than two days.

*M. cerebralis* has been experimentally passed with the faeces of the great blue heron, but there are no documented reports that transmission has occurred naturally via this route. There is no evidence that this parasite can be transferred within eggs (Markiw

1991). However, viable spores might be carried on the egg surface or with egg-packing materials.

*2) Methods of prevention and control.* Several approaches have been used to control whirling disease, including husbandry techniques, disinfection and water treatments. However WD spores retain their viability for many years even following periods of drying and freezing and are generally resistant to disinfectants. Hence, most approaches for the prevention of WD in a salmonid hatchery are based on the premise that once a fish has reached a length of 5-6 cm (about six months), it will generally be resistant to infection. At this age sufficient ossification of the skeleton should have taken place to reduce the chance of spores becoming established. However, there are several reports of older fish becoming infected.

Where possible, earthen ponds or raceways should not be used for rearing fish considered susceptible to WD, but if used, the ponds should be drained annually and disinfected with either calcium oxide or sodium hypochlorite. In some countries, filtering the water through sand-charcoal filters has been successful. Current legislation of many countries requires the fish to have been reared in water known to be pathogen-free.

Hatchery managers should ensure that fish moved from other sources are certified free of WD. Recent work suggests that eyed eggs or one-day-old sac fry do not provide conditions suitable for WD development; thereafter fry are susceptible and should be reared in spore-free water (e.g., spring water). There is no known proven chemotherapy treatment for WD, although there are a few reports suggesting that partial prophylaxis may be achieved (i.e., reduction in spore numbers) following fumagillin treatments (El-Matbouli and Hoffmann 1991).

*Ichthyophthirius multifiliis.*

*1) Modes of expression in fish.* This parasite is distributed worldwide and is the causative agent of "white-spot" among freshwater fish. Under aquarium conditions it often becomes prevalent. Predominant signs of infection include white spots on the body surface, which represent the trophozoite or feeding stage of *Ichthyophthirius* beneath the epidermis. Trophozoites lie within a cavity and move by means of cilia in a typical rolling motion. Microscopical examination of skin scrapes allow the characteristic horseshoe-shaped nucleus to be seen. As the trophozoite matures it breaks through the epidermis and encysts on a suitable substrate and undergoes mitosis to produce free-swimming tomites, which are then able to reinfect other hosts. The rate of reproduction is temperature-dependent. The host response includes an acute to subacute hyperplasia, which may continue with pronounced necrosis.

*2) Methods of prevention and control.* Treatment is directed towards the free-swimming trophonts using formalin, malachite green or both, and is generally successful if repeated with three treatments on alternate days.

*Kudoa.*

*1) Modes of expression in fish. Kudoa thyrsites* affects the muscle of many marine fish where the parasite grows and produces proteolytic enzymes that break down the filaments of the muscle fibres. The effect of *Kudoa* on the fish product has mainly been seen in relation to fish which are sold cold-smoked. Affected fish show distinct white marks over the surface of the fillet, which may advance into a lysed soft flesh resulting in subsequent rejection by processors. In Atlantic salmon the host response is delayed until the mature parasite ruptures, releasing an immense number of spores into the interstitial spaces between the muscle fibres (Harrell and Scott 1985).

*2) Methods of prevention and control.* It has been suggested that the proteolytic effect of *Kudoa* may be controlled by rapid chilling and processing after capture. In addition, the prevention and treatment with various drugs may be possible. However, further knowledge of the life cycle, possibility of intermediate hosts, and knowledge of infective stages is likely to be more valuable in the short-term.

*Hexamita.*

*1) Modes of expression in fish.* Two species of the flagellated parasite *Hexamita, H. intestinalis* and *H. salmonis,* affect the gall bladder and upper intestine, including the pyloric caeca, in young trout and salmon in fresh water and other aquarium fish in the warm period of the year. Characteristic gross signs include dark colour, faecal pseudocasts, anorexia, poor growth and severe cranial ulcerative lesions. Recently a systemic infection by a hexamitid resembling *H. salmonis* has been described from chinook salmon weighing over 1 kg in seawater (Kent et al. 1992) and Atlantic salmon in sea-cages (Poppe et al. 1992).

In fresh water the significance of this genus has been disputed. In some cases infection has resulted in a catarrhal enteritis whereas other outbreaks are not associated with any pathological changes. In contrast, Kent et al. (1992) considered that this parasite represents a significant threat to farmed chinook salmon with high mortalities and ease of transmission between fresh and salt water. Affected fish showed anaemia, oedema, inflammation and congestion of the liver.

*2) Methods of prevention and control. H. salmonis* is a typical opportunistic pathogen and management practices, including adequate water changes with the removal of organic debris, are important in preventing infestation by this parasite. An improvement in nutrition combined with vitamin C supplements has also been reported as beneficial.

*Paramoebic Gill Disease.*

*1) Modes of expression in fish.* Mortalities of sea-caged salmonids in Tasmania, Australia have been caused by an amoeboid protozoan, *Paramoeba* sp., primarily in the summer during increasing water temperatures. Large numbers of the parasite are associated with a severe gill branchitis, necrosis and epithelial hyperplasia (Roubal et al. 1989).

2) *Methods of prevention and control.* Control is achieved by bathing the fish in fresh water for several hours.

*Trichodina Species.*

1) *Modes of expression in fish.* The parasite occurs in marine and freshwater culture. It possesses an immediately recognisable ring of internal denticles and a complex conspicuous oral ciliature whereby it moves over the surface of the gills and skin. Infected fish may show a greyish sheen on the body surface due to excess mucus production resulting in a sub-acute lesion with associated hyperplasia. Mortalities have been attributed to *Tricodina* infections among coho salmon in the U.S. (Sindermann 1977).

2) *Methods of prevention and control.* The maintenance of good water quality in tanks and ponds is often sufficient to prevent this parasite establishing in large numbers.

*Henneguya.*

1) *Modes of expression in fish.* At present, around six species of *Henneguya* (Myxospora) have been described from channel catfish (*Ictalurus punctatus*) occurring on the body and gills. Only the interlamellar forms of this disease are considered to cause severe pathology. However other species, e.g., *H. salminicola*, parasitic in brown trout and Atlantic salmon, and *H. zschokkei* in sockeye salmon (*Oncorhynchus nerka*), are often responsible for a milky-flesh condition. The genus is recognized by the characteristic tadpole-shaped cysts which contain two relatively small polar capsules.

2) *Methods of prevention and control.* No treatment is currently available, although increased aeration of the water has been shown to be beneficial.

*Cryptobia.*

1) *Modes of expression in fish.* This biflagellate commonly parasitizes the blood of freshwater salmonids, particularly *Oncorhynchus* spp., during spawning, but also occurs in juvenile rainbow trout on the west coast of North America. Infection results in a severe anaemia, exophthalmia and abdominal distension with mortalities in some stocks. The parasites are reported to be transmitted by the leech, *Piscicola salmositica* (Mann 1962).

2) *Methods of prevention and control.* Treatment specifically against *Cryptobia* is not well developed. There is some evidence that innate resistance to the parasite occurs in some Pacific salmon stocks that are in contact with this protozoan (Bower and Evelyn 1988).

*Dermocystidium.*

*1) Modes of expression in fish.* The gill epithelium and skin are considered typical sites of infection for *Dermocystidium* sp.; however, systemic infections of salmonids have been reported (McVicar and Wootten 1980; Nash et al. 1989). Small cysts about 1 mm in diameter occur on the gills and may contain a large number of unicellular organisms. In fry, disease outbreaks involving the gills can physically prevent the gill covers from closing, hence mortalities can be very high. The host may be able to contain a small number of spores by forming a fine fibrous capsule around them; however, it appears many spores enter the blood system and quickly infect all tissues. In larger fish, rounded glistening white cysts have been reported on the gills as well as the gut mucosa. The multinucleate cells in the internal organs may represent a developmental stage in the life cycle of *Dermocystidium.*

*2) Methods of prevention and control.* Sources of infection and modes of transmission are currently too poorly understood to offer advice on methods of control.

## METAZOAN PARASITES OF SALMONIDS

### *Biology and Classification of the Main Groups Significant to Salmonid Culture*

The metazoan parasites are multicellular organisms expressing both ecto- and endoparasitic life styles causing significant pathology and mortalities. These parasites are represented in several groups including monogenetic and digenetic trematodes, cestodes, nematodes, acanthocephalans, leeches, molluscan larvae and crustaceans. The important metazoan parasites of salmonids are summarized in Table 5.

*Monogenea.* Monogenean (trematodes) are generally small, ranging up to a few millimetres in length. They have a direct life cycle and many are host specific. Ciliated free-swimming larvae called oncomiricidia hatch from relatively large eggs and must then infect a suitable host within a short period in order to mature. However, gyrodactylid trematodes produce viviparous young. Based on the morphology of the posterior attachment organ (haptor or opisthaptor), two major groups are distinguished: Monopisthocotyle (e.g., *Gyrodactylus* and *Gyrodactyloides*) and Polyopisthocotyle (e.g., *Discocotyle*). In general, further classification will include reference to morphological characters including the number of marginal hooks, haptor, hamuli and the morphology of the testis and ovaries (Beverley-Burton 1984). Malmberg and Malmberg (1993) have underlined the importance of complementary morphometric, experimental infection investigations and DNA analysis in order to help distinguish natural and "cultured" populations of similar *Gyrodactylus* species on salmon.

*Digenea.* The life cycle of digeneans is often complex. The free-swimming miracidium hatch from the eggs to infect the first intermediate host which is a species of

**TABLE 5**

Important metazoan parasites of salmonids.

| Genera | Class or group | Location in fish |
|---|---|---|
| *Acanthobella* | Hirudinea | Skin |
| *Acanthocephalus* | Acanthocephala | Intestine |
| *Anisakis* | Nematoda | Musculature |
| *Argulus* | Crustacea | Skin, fins, gills |
| *Bulbodacnitis* | Nematoda | Intestine |
| *Contracaevum* | Nematoda | Intestine |
| *Cotylurus* | Digenea | Heart |
| *Crepidostomum* | Digenea | Gall bladder, liver |
| *Cystidicoloa* | Nematoda | Swim bladder |
| *Dactylogyrus* | Monogenea | Gills |
| *Diphyllobothrium* | Cestoda | Intestine |
| *Diplostomum* | Trematoda | Eyes |
| *Echinorhynchus* | Acanthocephala | Intestine |
| *Ergasilus* | Crustacea | Skin, fins |
| *Eubothrium* | Cestoda | Intestine |
| Glochidia larvae | Mollusca | Gills |
| *Gyrodactylus* | Monogenea | Gills, skin, fins |
| *Illinobdella* | Hirudinea | Gills |
| *Lepeophtheirus* | Crustacea | Skin |
| *Lernaea* | Crustacea | Skin, fins |
| *Piscicola* | Hirudinea | Skin, fins |
| *Pseudoterranova* | Nematoda | Muscle |
| *Salmincola* | Crustacea | Gills |
| *Sanguinicola* | Digenea | Heart, gills |

snail (family Lymnaeidae), within which they undergo a phase of asexual reproduction in the liver tissue. Eventually the free-swimming cercariae are released which may either infect fish as final hosts where they mature into adult trematodes, or infect fish as intermediate hosts, often through the gill region, to form the metacercarial stage. In the latter case, completion of the life cycle occurs when the infected fish is eaten by a suitable final host, e.g., birds. Although the metacercariae may display many of the taxonomic features of the adult, they usually lack developed reproductive organs. Hence classification is often carried out on the adult by examining the ventral holdfast organ, location of the genital pore, and the presence of cephalic structures around the oral cavity (Schell 1985). Genera important in salmonids include *Sanguinicola*, *Cardicola* and *Diplostomum*.

*Cestoda*. In general, cestodes or tapeworms affecting fish have a life cycle involving more than one host. Fish may act as final or intermediate hosts. Eggs are released in the faeces, and either the eggs or the hatched larvae (coracidium) are eaten by an invertebrate host where they penetrate the gut wall to undergo further development. For maturation to occur in fish, the procercoid must be eaten by another suitable host to continue its development. In some cases the plerocercoid (larval) stage develops in the fish (e.g., *Diphyllobothrium*) and development only continues when eaten by the definitive host, e.g., bird, carnivorous fish.

Two sub-classes are distinguished, the more primitive Cestodaria and the Euscestoda. In the Cestodaria no scolex is present. Classification of mature cestodes is based on the characteristics of the scolex and the organs within the mature proglottid, whereas immature cestodes are classified on a general morphological basis (Overstreet 1978).

*Acanthocephala*. The Acanthocephala (spiny-headed worms, e.g., *Echinorhynchus* spp.), are worms occurring in both fresh and marine environments. Hooks on a retractable armed proboscis become embedded in the intestinal tract of their host. Each member of this group requires an invertebrate host, usually an arthropod, to complete their life cycle. The egg contains an acanthor larvae, which is released when ingested. Following its development into a cystacanth the parasite will develop to a mature worm if consumed by the final host. Paratenic hosts, where the immature acystacanth encysts within the mesentery without further development, contribute to the completion of the life cycle by providing a link between an intermediate and final host. The characteristics of the proboscis, presoma and other internal structures are used to identify the adult worms (Yamaguti 1963*a*).

*Nematoda*. Most nematodes are cylindrical, elongate worms of the intestinal tract occurring in freshwater and marine fishes. The eggs are passed with the faeces of the host and hatch into free-swimming larvae generally to be eaten by an intermediate host, e.g., arthropods. Other groups produce viviparous larvae. Many develop to adult forms in a suitable fish host, although some species only use the fish as an intermediate host and will mature in the intestinal tract of a suitable final host, including mammals, other fish or birds. Genera important to salmonids include *Anisakis*, *Pilonema*, *Contracaecum*, *Bulbodacnitis* and *Cystidicola*. The classification of nematodes relies upon identifying external and internal anatomical features (Anderson and Bain 1982).

*Mollusca*. Mature freshwater bivalve molluscs, in particular members of the family Unionidae, release large numbers of larvae known as glochidia, which are obligate larval parasites on the gill lamellae of fish (Bruno et al. 1988).

*Crustacea*. There are two subclasses of Crustacea that contain parasites for salmonids, Branchiura (e.g.,*Argulus*) and Copepoda (e.g., *Lepeophtheirus*). Some of these are economically important in freshwater and marine aquaculture throughout the world. The classification of parasitic crustaceans is based on the body segmentation, the form of the attachment organ, morphology of the egg sac, and number and structure of the limbs (Yamaguti 1963*b*).

The Branchiura are dorso-ventrally flattened, obligate ectoparasites of the skin and fins. Most belong to the genus *Argulus*. The juveniles are morphologically similar to the adults, possessing a proboscis-like mouth for feeding, and curved hooks and suckers that serve as organs of attachment.

The Copepoda are considered the most important ectoparasitic group on fish, particularly as they may act as intermediate hosts for other diseases (Mulcahy et al. 1990). Four families, Ergasilidae, Lernaepoidae, Lernaeidae and the Caligidae, contain most of the marine and freshwater copepods important to salmonid aquaculture. The Ergasilidae are parasitic on the gills or buccal cavity of freshwater fish where they are found attached by specialized prehensile hooks. *Ergasilus caeruleus* has been reported from rainbow trout and three other species have been reported from sockeye salmon.

Most of the parasitic members of the Lernaepoidae belong to the genus *Salmincola* (gill maggots), which are parasitic on salmonids including Atlantic salmon, brook trout and Arctic grayling (*Thymallus arcticus*) in fresh water. *S. salmoneus* is host-specific to Atlantic salmon and is well adapted to the anadramous life cycle of salmon (McGladdery and Johnston 1988). Free-swimming larvae locating a host attach to the gill tissue by means of a filament and second maxillae forming a permanent structure termed the bulla.

The Lernaeidae are best represented by the genus *Lernaea*, the "anchor worms", which mainly affect freshwater fish worldwide including salmonids. The head of the female parasite is modified into a branched anchor-shaped structure with cephalic horns and becomes embedded in the body wall of the host.

Parasitic copepods belonging to the family Caligidae are common external crustacean parasites of wild and farmed fish in the marine environment, and include the genera *Caligus* and *Lepeophtheirus*. The former is a large non-specific genus of over 200 species affecting salmonids and many other fish groups. In contrast, fewer species of

*Lepeophtheirus* have been described, with *L. salmonis* as a significant parasite of sea-farmed *Oncorhynchus*, *Salmo* and *Salvelinus* species and the cause of serious losses to commercial production. The life cycle of caligid copepods, including *L. salmonis* and *C. elongatus*, consists of 10 stages: two free-living, planktonic naupliar stages, one free-swimming copepodid stage, four attached chalimus stages, two pre-adult stages and an adult stage. Fish are infected by the copepodites present in the upper water column or by adults that may move from one host to another (Bruno and Stone 1990).

*Hirudinea*. The phylum Annelida contains the class Hirudinea (leeches), of which several genera, including *Piscicola*, *Acanthobdella* and *Illinobdella* spp., have been recorded as ectoparasites from a variety of salmonids. However it is likely that there is little host specificity within these groups of parasites. Each animal has a distinct sucker at each end of a flattened or tube-like body.

### *Modes of Expression and Treatment of Metazoan Parasites*

*Gyrodactylus salaris (Monogenea).*

*1) Modes of expression in fish.* *Gyrodactylus salaris* was first reported on wild Atlantic salmon in Norway by Johnsen (1978), and has been held responsible for the severe reductions in the number of salmon parr and subsequent catches of returning mature salmon in infected Norwegian rivers. Affected fish may show frayed fins, damaged gills, loss of skin epithelium with localized or severe dermatitis, hyperplasia and an increase in goblet cells due to the feeding activity of the parasite and the action of the marginal hooks. Gyrodactylids are viviparous with a brief reproduction cycle, which may result in the development of epizootics within a short period of time. Mortalities are attributed to disruption of normal osmotic properties of the skin and infection by secondary pathogens.

*2) Methods of prevention and control.* Salmonids susceptible to *G. salaris* are readily infected when the parasite is given the opportunity to transfer between individuals and heavy parasite loads will result in mortalities. At the present time this parasite is absent from the British Isles (Bakke and MacKenzie 1993), but present in Scandinavia and continental Europe. Current U.K. legislation aims to prevent the introduction of this parasite by ensuring any imports of live salmonids are from areas free of *G. salaris*. Outbreaks in tanks and ponds will necessitate appropriate treatment, and formalin or potassium permanganate baths are recommended. In addition procedures to prevent the introduction of the parasite must be employed and supported by the maintenance of healthy stock.

*Digenea.*

*1) Modes of expression in fish.* *Diplostomum* is one of a number of strigeoid metacercariae that are generally located in the lens of freshwater fish worldwide. *D. spathaceum* is transmitted by seagulls and parasitizes rainbow trout during the summer months. Infestation may first appear as localized swelling, or red patches on the fins and around the orbital region, resulting from the cercariae penetrating and rupturing the blood capillaries. The larvae migrate to the eye, often in large numbers, resulting in the formation of a subcapsular cataract and blindness (Shariff et al. 1980), pronounced subacute inflammatory reactions, exophthalmia, liquefaction of cortical fibres, necrosis and localized haemorrhaging. Salmon appear to be relatively resistant to these eyeflukes.

Only a few blood flukes have been described from salmonids and include *Sanguinicola klamathensis*, *S. davisi* and *Cardicola alseae*. These digeneans have been reported from rainbow trout hatcheries in North America. Affected fish may exhibit a grey-slimy appearance and become lethargic.

Various species of *Phyllodistomum* dwell in the urinary bladder and ducts of freshwater and marine fish and have been occasionally reported in rainbow trout, brook trout and Arctic grayling.

*2) Methods of prevention and control.* The disruption of the life cycle of *Diplostomum* and other digeneans, where possible, provides the best means of controlling diplostomatosis and other digenean infections. The elimination of the snail host appears the most effective means of controlling the disease, and may be achieved by draining and cleaning ponds, which is a generally labour-intensive process, or through the application of a molluscicide. The snails prefer slow-moving water with some vegetation on which to live and feed; therefore ponds or channels where weed growth has occurred should be cleared. Both the enclosure of spring-water sources and the employment of good management practices are also recommended. In a few cases ultraviolet light has been used to kill the metacercariae. In areas where raw fish is used as a fish feed, control of digenean parasites is difficult.

*Cestoda.*

*1) Modes of expression in fish.* Several species of *Diphyllobothrium* cestodes have been noted as parasites of salmonids in fresh water in the wild and, in a number of cases, of cultured salmonids, and include *D. cordiceps*, *D. ditremum* and *D. latum*. These generally occur in the liver, heart mesentery and intestine as plerocercoid larvae. In the host, granulomas, necrotic myofibrils and oedema develop near or around these parasites. The plerocercoid becomes encapsulated but continues to develop until it can break from the cyst and becomes free within the abdominal cavity. Heavy infestations are likely to retard growth. Rodger (1991) suggested that the high prevalence of *D. ditremum* in farmed Atlantic salmon resulted in a cumulative mortality which exceeded 30% from time of stocking to smoltification. Humans and other mammals are well-documented definitive hosts for *Diphyllobothrium* (Ohbayashi et al. 1977).

The genus *Eubothrium* has been reported from species of salmon, grayling and trout where they occur in the pyloric caeca or intestine as adult parasites. In most studies little evidence of physical damage to the fish has been reported, although several reports show infection by *E. salvelini* to be associated with haemolytic anaemia, reduction in growth and the fish's ability to adapt to seawater. Infections of the vitreous humor of the eye of chinook salmon smolts by *Gilquinia squali* have been recorded resulting in a 10% mortality (Kent and Margolis 1991).

*2) Methods of prevention and control.* There is no reported effective treatment for *Diphyllobothrium* or other cestode infestations in salmonids.

*Acanthocephala.*

*1) Modes of expression in fish.* Several genera including *Echinorhynchus*, *Neoechinorhynchus* and *Acanthocephalus* have been reported as parasites of rainbow trout and Atlantic and Pacific salmon in the U.S. and Europe. When present in the gut in large numbers, this group is considered to cause a serious effect as the parasites can completely obstruct the gastrointestinal tract. Bullock (1963) has reported *A. jacksoni* as highly pathogenic for cultured trout as each worm can reattach in the gut several times, resulting in haemorrhagic necrotic ulcers accompanied by an intense inflammatory reaction.

*2) Methods of prevention and control.* No effective treatment is available for infections with this group of parasites, which often go undetected until post-mortem.

*Nematoda.*

*1) Modes of expression in fish.* The class Nematoda consists of many species of bilaterally symmetrical organisms that have a pseudocoelom and a gut. The pathology associated with nematode infestations in salmonids is variable. In general, nematodes present in the intestinal tract do not appear to cause extensive pathologic consequences, e.g., *Anisakis*, *Cystidicoloides*, although this is considered difficult to ascertain. However, migrating larvae cause considerable damage in many organs, as do larvae of the genus *Spiroxys* and *Contracaecum*, which are generally found in the body cavity.

Both *Anisakis* and *Contracaecum* are common in wild salmonids in seawater and may be present in large numbers. The development to adult worms occurs in a final host, and depending on the species of nematode, these may be mammals including humans, birds, or other fish.

Ingestion of third-stage larvae of *Anisakis simplex*, the most common species, and other anisakid nematodes, can cause disease in fish and humans worldwide and is termed anisakiasis. The commercial value of fish infected by this nematode is considerably reduced. Although this parasite is not considered a serious pathogen in marine fish, infestation in freshwater fish may result in haemorrhage through the bursting of blood vessels and thrombus formation. In humans the larvae do not mature, but cause extensive lesions in the intestine or stomach.

*2) Methods of prevention and control.* The presence of *Anisakis* infections in humans can be prevented by deep freezing or adequate cooking. In a similar manner, marine waste fish used as a food substance for freshwater fish should be avoided, thus preventing the parasite infecting these fish. There are no adequate chemical treatments for infestations in fish.

*Mollusca.*

*1) Modes of expression in fish.* Each year bivalve molluscs such as the freshwater pearl mussel, *Margaritifera margaritifera*, release numerous glochidia which are parasitic on the gills of the Salmonidae and other fish species. The glochidia reach the gills passively and attach to the fish host where they undergo metamorphosis into juvenile molluscs. Attached glochidia become covered by a hyperplastic, moderately granulomatous response proliferating to involve adjacent gill lamellae. The glochidia, which have attached at the gill filament tip may slough away with some localized haemorrhaging (Bruno et al. 1988). After several months of development, each juvenile mollusc will naturally detach. Meyers and Milleman (1977) have suggested that mortalities only occur when glochidia numbers are very high.

*2) Methods of prevention and control.* Various chemical treatments to remove pearl mussel glochidia from the gills of Atlantic salmon have been unsuccessful. Avoidance of the glochidia appears the best option. In the spring the glochidia will detach naturally.

*Crustacea.*

*1) Modes of expression in fish.* *Salmincola* species are small, freshwater parasites on the gills of salmonids in Europe, Asia and North America, which may occur in large numbers on individual fish. The parasite settles on the gill lamella or at the base of fins making them congested and frayed.

There are numerous species of *Lernaea* reported from freshwater fish worldwide. Although they can infect salmonids there are relatively few reports in the literature, probably due to the relatively high temperature at which the parasite develops. Each female parasite is permanently fixed to the muscle tissue of the host by an anchor-shaped head, and in some cases will even penetrate into the body cavity. Young fish are particularly susceptible and become lethargic with weight loss and secondarily infected by bacteria or fungi.

*Lepeophtheirus salmonis* and *Caligus elongatus* are copepod parasites of wild and farmed salmonids, which have become a serious problem in the marine culture of salmonids. Attached copepodids, chalimus larvae, pre-adults and adults feed on host mucus, blood and skin, which results in skin erosion particularly on the head, back and perianal region. These lesions develop without treatment into a deeper necrotising and ulcerative dermatitis, with sub-epidermal haemorrhage and exposure of the cranial bones.

Apart from secondary pathogens gaining easy access to these lesions, *L. salmonis* has also been reported as a direct vector of other pathogens (Nese and Øivind 1993).

*2) Methods of prevention and control.* Organophosphate insecticides are the most common treatment for the genera *Argulus* and *Lernaea*, and this may be supplemented by the use of ultraviolet sterilisation of water supplies to kill larvae. At the present time the control of *Lepeophtheirus* and *Caligus* in aquaculture is largely dependent upon the use of an organophosphorus pesticide, dichlorvos. However, because dichlorvos represents a human health risk and may have detrimental side-effects on the environment, and cases of resistance among populations of sea lice have been recorded, more acceptable forms of control are currently being investigated (*see* Chapter 16).

Current strategies are concentrating on: (1) pesticides that are effective against all stages of the lice, (2) the use of cleaner wrasse (Labridae) which feed on lice from the body surface of infected fish, (3) hydrogen peroxide treatments (Roth et al. 1993; Bruno and Raynard 1994), (4) fallowing (Bron et al. 1993), and (5) vaccine research (Reilly and Mulcahy 1993). To complement these studies, a thorough understanding of the fecundity, population dynamics and general biology of the lice is required (Tully 1989).

*Hirudinea.*

*1) Modes of expression in fish.* Leeches are segmented worms that attach periodically to freshwater and marine fish species generally without host specificity and take a relatively large blood meal. The pathologic consequences induced depend upon the frequency of feeding, blood loss and the level of infestation, but may include an ulcerative dermatitis with haemorrhage and epidermal hyperplasia. Fish may also become infected by secondary pathogens as well as other disease agents transmitted directly by the leech, such as infectious hematopoietic necrosis virus (Mulcahy et al. 1990) and other parasites including *Cryptobia* and *Haemogregarina*.

*2) Methods of prevention and control.* It is difficult to control leeches in cage culture. In ponds they are easily killed by draining.

## TECHNIQUES OF DIAGNOSIS

### *Gross Symptoms*

*External Examination.* Necropsy of moribund fish is an essential step in diagnosing disease and must be carried out in an organized and systematic manner. At least five fish exhibiting typical symptoms of a disease should be selected. Before sacrificing live fish, behavioural patterns should be noted. An anaesthetic agent, such as ethyl-p-aminobenzoate or tricane methanesulphonate, dissolved in water is the recommended method of killing fish (*see* Summerfelt and Smith 1990).

In each case the general condition of all external surfaces of the animal should be recorded, paying particular attention to raised or lost scales, areas of discolouration, parasites, location of any lesions, suggestion of skeletal deformity or muscle atrophy. The eyes should be examined for cloudiness of the cornea, degenerative cataract or exophthalmia. Any signs of blood, exudate or oedema in the anterior chamber of the eye must also be recorded. All fins should be examined for evidence of erosion, fraying, necrosis or small coloured spots or raised areas. Both the normal skin surface and the margins of any lesion should be examined and bacteriological samples taken from developing wounds, rather than from larger lesions as these are subject to secondary contaminants. Swabs should be plated aseptically onto an appropriate culture medium for later bacteriological examination. Fresh skin scrapes should be examined microscopically for bacteria or fungal hyphae. Samples from the edge of lesions may be fixed for light microscopy.

The mouth should be opened and the oral cavity viewed, noting any discolouration, raised areas or parasites. Any redness or swelling around the abdomen should be recorded. Faecal material, if present, should be examined microscopically. After this external examination, the weight and fork or total length of the fish should be noted together with a detailed history, which will include the name of the farm, group and year class, water temperature, percentage mortality, first observation of disease symptoms and any recent treatments or handling.

*Internal Examination.* Lay the fish on its right side to allow better access to the spleen. If required, a blood sample should be taken at this stage. Tissues should be examined, records kept, and then each tissue removed carefully in turn. A portion should be fixed for light microscopy in formol saline. After dissecting out the eye, examine the orbit for any haemorrhage or exudate. Remove sufficient amount of the cranial bone to fully expose the brain and then cut the neural connections. Remove the brain and note any haemorrhage or other discolouration of the cerebrospinal fluid or the organ itself. The brain rapidly undergoes autolysis and therefore it should be fixed as soon as possible.

Next, examine the gills by cutting or lifting the operculum and recording colour, haemorrhage, excess mucous or parasites. A wet mount should be examined for evidence of parasitic infection. The body cavity should be opened and all internal organs exposed. Make the incision starting from the operculum, cutting through the pelvic girdle along the mid-ventral line, through the pectoral girdle and on to the anus. The contents of the abdominal and pericardial cavities are now clearly seen.

The presence and colour of any fluid in the abdominal cavity should be noted and samples plated for bacterial culture. Each organ should be examined taking care to note abnormality with respect to size, colour or consistency and how much adipose fat is located between the organs. Before removing organs small pieces of kidney should be removed for virology testing. Then bacteriological samples should be taken with a sterile loop from tissues appearing abnormal. During the dissection portions of each organ should be fixed in formol saline for light microscopy. Small fry can be fixed whole with a cut made along the ventral surface to expose the viscera to the fixative.

Lift the cranial border of the bulbous arteriosus and cut the connection to the ventral aorta, this enables the heart to be moved sufficiently to expose and sever the sinus venosus. The heart should be examined for parasites or lesions associated with the myocardium. Most of the abdominal organs can then be removed by cutting the oesophagus and by holding the cut anterior end of the gut with forceps, lifting and dissecting the intestine towards the anus. In this manner the liver, gonads, spleen and intestinal tract are likely to be removed in their entirety from the fish. The liver can now be dissected and examined, noting any abnormal colour of a cut surface, together with the appearance of the bile in the gall bladder. Bile can be sampled using a syringe or vacutainer and frozen for later examination. If the spleen is swollen, then material for bacterial culture should be taken. A similar examination of the caecal area, stomach and intestine should be carried out. The lumen should be cut open, noting any abnormality of the mucosal surface. Scrapings can be prepared for microscopic examination. The swim bladder is normally white or a clear hollow organ, and exudate or haemorrhagic areas should be recorded and sampled.

The kidneys lie between the spinal column and swim bladder. Both the cranial and caudal areas should be sampled for light microscopy and notes made on colour, regions of swelling, grey mottled appearance, haemorrhage, exudate or consistency.

Necropsies are vitally important in the diagnostic process, but in many cases more sophisticated laboratory examinations, such as viral examination, bacteriology, ELISA and serologic identification, will be necessary to identify the aetiological agent. In the case of bacterial diseases it is necessary to determine the antibiotic resistance spectra so that the appropriate antibiotic can be chosen to treat the disease.

Notes taken at the necropsy, together with histopathology, bacteriology and virology data on the fish, provide a wealth of information and assist in diagnosis of the nature and cause of a particular disease. Photographic information also complements such diagnostic reports. Additional supporting data will come from the farm's case history and provide information on disease trends and changes in antibiotic resistance patterns. A standard necropsy form using standardized and consistent descriptors, such as used by most fish health specialists, is strongly recommended and will greatly enhance the value of the database for future reference.

### *Microscopic Examination of External Organs*

Changes in the skin are the most readily observed clinical features of fish as the surface is vulnerable to damage from a variety of sources including parasites, bacterial and fungal infections, polluted waters, handling and net damage.

Dead or anaesthetized fish should be immersed in clean water and the fins examined under a stereomicroscope at a magnification of 15-20×. Entire fish or removed tissues such as the gills, eyes, anus and skin with any parasites should be fixed in 70% alcohol and sent to the laboratory for identification.

### *Histopathology*

*Light Microscopy.* Histology deals with the microscopic appearance of tissues. Tissues can be considered normal if the observations are those commonly found in the given species, sex and age group of the species living in the same environment. Histological reports provide the basis for the understanding of physiological and pathological processes. Soon after death, tissues begin to undergo post-mortem changes not associated with any particular pathogen. These changes can be divided into putrefactive, which result from the invasion of the tissue by bacteria from the alimentary tract, and autolytic changes, which are due to the action of enzymes from dead cells. Both of these changes may be retarded by low temperatures or prevented by the use of chemical fixatives.

Fixation may be described as a procedure that will preserve the structure, shape, relationship and chemical constituents of tissues and cells after death. Hence, the purpose of fixation is to preserve the structural organization of the tissue. There are two main types of fixation, chemical and freezing (i.e., physical fixation).

A good chemical fixative will prevent bacterial and enzymatic digestion, insolubilise tissue components to prevent diffusion, and protect against damage from subsequent steps in tissue processing. Chemical fixation may cause changes in the composition and fine structure of the tissues resulting in staining artifacts during histochemical procedures. The structural changes may result from denaturing and cross-linking certain tissue proteins. Therefore, adequate fixation is the foundation for the subsequent stages in the preparation and analysis of tissue sections. The importance of proper fixation cannot be over-stressed. If fixation is unsatisfactory interpretation of the tissue section will be difficult or even impossible.

The most common fixing agent for light microscopy is formaldehyde (which is supplied as a 40% concentrate, i.e., formalin). In this acidic form it is unsuitable as a histological fixative and will require dilution and the incorporation of buffering agents to maintain a slightly alkaline pH. The following formula has been developed especially for fish tissues and is conveniently prepared in batches of 2.5 litres (Table 6). A formol saline fixative permits the application of a variety of staining procedures in order to render the constituents of the tissue more readily visible. Both cytological detail and red blood cells are well preserved.

**TABLE 6**

Recommended fixative for fish tissue.

| **Buffered formol saline** | |
|---|---|
| 40% formaldehyde (formalin) | 500 ml |
| Sodium dihydrogen orthophosphate $NaH_2PO_4.2H_2O$ | 22.75 g |
| Sodium chloride NaCl | 22.50 g |
| Disodium hydrogen orthophosphate $Na_2HPO_4$ | 32.50 g |

Make up to a final volume of 2.5 litres with distilled water

The rate of penetration of fixatives into tissue is important, and as this process is relatively slow (8-24 h), each block of tissue selected from the freshly killed fish should not exceed 1 $cm^3$. Each tissue should be carefully dissected from the fish to avoid tearing or excessive bleeding, and cut using a clean sharp scalpel. The volume of fixative used should be at least 20 times the bulk of the tissue to be fixed. Tissues containing bone, e.g., gills, skin, generally require decalcification before further processing. Tissues are then gently dehydrated to remove aqueous fixative and any tissue water "cleared" with a substance totally miscible with both the dehydrating fluid and the embedding agent, which follows this step. Tissues are blocked in molten wax before sectioning. The wax is then removed from each section, which is rehydrated and then stained. Haematoxylin and eosin is used for routine diagnostic work, supported by other staining procedures such as Gram's, periodic acid-Schiff reaction (PAS), Giemsa and trichrome methods.

The use of a cryostat to cut frozen or previously fixed tissue sections for the subsequent identification of fats, enzymes and other heat or labile substances is a routine procedure in diagnostic laboratories.

*Electron Microscopy.* The use of transmission electron microscopy allows for the study of sub-cellular morphology and as an adjunct to light microscopy. For transmission electron microscopy small pieces of tissue ($<2$ $mm^3$) from freshly dissected fish are commonly fixed in neutral buffered glutaraldehyde at 4°C. This fixative is seldom used alone and is often followed by treatment of tissues in buffered osmium tetroxide, which when reduced acts as an electron-dense stain that reacts principally with lipids. The majority of embedding media fall into one of four main groups: (1) acrylic media, (2) epoxy resins, (3) polyester resins, and (4) water-soluble media. Thin sections (60-90 nm) are cut using an ultramicrotome.

Two useful stains that may be used either before or after embedding are phosphotungstic acid and uranyl acetate. Staining is usually carried out after mounting the sections upon a grid and staining the whole grid. The absorption of stain is rapid and a few minutes is usually sufficient.

Scanning electron microscopy uses solid pieces of tissues rather than sections and allows the perception of three-dimensional (3D) views of the surface of cells or tissues. A beam of electrons scans the sample surface and the reflected electrons are collected by a detector and displayed as a 3-D image.

### *Bacterial Culture*

The management of bacterial outbreaks relies on correct identification and the *in vitro* testing of antimicrobial agents. Bacterial cultures should only be taken from recently killed fish. Rapid post-mortem changes take place shortly after death, with invasion by environmental or gut microbes, which may completely mask the causative disease agent. Where possible several fish should be examined including those exhibiting early stages of the disease.

Material for culture is taken with a sterile loop or swab and inoculated onto a nutrient medium appropriate for the suspected disease agent. In many cases this will be a non-selective medium capable of supporting the growth of many pathogens, although additional selective media may also be included. Initial sampling should begin with the external surface, particularly the gills and obvious lesions. Faecal bacterial cultures can be taken by inserting a small loop into the anus. Internally, cultures should be taken from body fluids and from one or more organs, in particular the kidney, spleen and liver. Incubation period and temperature will vary and are largely influenced by the optimum growth temperature of the suspected bacterial pathogen.

Colony morphology, biochemical tests and the use of diagnostic keys will assist with identification (Austin and Austin 1987). Gram's stain is the most valuable method for differentiating micro-organisms. Bacteria that do not retain the primary violet but retain the counterstain are termed Gram negative, whereas bacteria which retain the primary violet in Gram's stain are termed Gram positive. Gram-negative pathogens can be divided initially into long filamentous bacteria belonging to the family Cytophagaceae (e.g., *Cytophaga psychrophila*) and shorter bacilli belonging to the families Enterobacteriaceae, Pseudomonadaceae and Vibrionaceae. Bacteria in the Cytophagaceae group are pigmented and show gliding motility, whereas the other groups are classified following various tests, e.g., those which show cytochrome oxidase activity such as *Aeromonas salmonicida*, most *Vibrio* spp. and *Pasteurella piscicida*. Motility, culture requirements, pigment formation and vibriostat testing will enable these and other bacteria in the bacilli group to be classified further. Cytochrome oxidase-negative bacteria include *Yersinia ruckeri* and *Edwardsiella tarda* and in this case may be separated by the production of indole and by other tests. Gram-positive pathogens, including *Renibacterium salmoninarum*, *Carnobacterium piscicola*, *Mycobacterium* spp., and *Streptococcus* spp. are distinguished by examining morphological detail, growth characteristics and biochemical properties.

### *Virus Isolation*

Salmonids are susceptible to a variety of viral infections but relatively few cause heavy mortalities and clinical pathology. Virulent viral diseases include infectious pancreatic necrosis (IPN), viral haemorrhagic necrosis (VHS) and infectious haematopoietic necrosis (IHN). The most common approach to diagnosis of these diseases is by culture of virus in tissue culture. In addition, histological data can provide important details on the pathology and host response of each animal. Appropriate host biopsy samples are homogenized, diluted and centrifuged to remove cell debris, filtered, and the material added to a growth medium in flasks containing on one side a tissue culture cell monolayer. The majority of viruses produce obvious degenerative changes in cultured cells, and such cytopathic effects (CPE) may be used for initial diagnosis. Confirmation of a specific virus is conducted using a specific antiserum either in a neutralisation of infectivity assay, or by immunostaining for virus antigens.

### *Indirect Determinations*

*Fluorescent Antibody Techniques.* An indirect fluorescent antibody technique (IFAT) for the detection of *R. salmoninarum* was first reported in 1975 by Bullock and Stuckey (1975) and followed in 1980 by a direct FAT method for the detection of this bacterium (Bullock et al. 1980). The latter approach was considered more sensitive than Gram staining, although the IFAT and DFAT approaches were not compared.

Both techniques have been used and comparative studies made to test their effectiveness compared with other diagnostic methods. False positives have been reported (Armstrong et al. 1989). With slight modifications including the use of DABCO (1,4-dianormala-bicyclo-[2,2,2]-octane) to retard fading of immunofluorescence and the use of Erichrome black T to reduce background fluorescence, IFAT has been used to examine ovarian fluids for *R. salmoninarum* (Elliott and Barilla 1986; Pascho et al. 1991). They suggest that the segregation of brood stock by this method can be used to reduce the prevalence of BKD in hatchery-reared salmon.

*Enzyme-Linked Immunosorbent Assays (ELISA).* Several enzyme-linked immunosorbent assays (ELISA) for the detection of *R. salmoninarum* have been developed (Pascho et al. 1991; Olea et al. 1993), which appear specific, quick and sensitive, and therefore offer advantages over culture which may take many weeks. Rapid ELISA methods for other fish pathogens have also been developed (Dixon and Hill 1983;

Vestergård Jørgensen et al. 1991). Important variations of ELISA utilising enzyme-linked antibody probes for specific pathogens include the dot blot and Western blotting. Dot blot assays have been used to identify viral pathogens, e.g., IPN (Melby and Christie 1994) and bacterial pathogens, e.g., *R. salmoninarum* by both dot blot (Sakai et al. 1987) and Western blotting (Griffiths and Lynch 1991).

*DNA Probes.* Over the last few years significant advances in the fields of molecular biology and immunology have had a major impact on the approaches used to diagnose infections in both research and diagnostic laboratories. In addition to the possession of specific antigens, all species contain portions of genetic material, (DNA or RNA), which are unique in terms of their sequence and can therefore be detected using species-specific gene probes. Fundamental to DNA probe-type assays are the definition of nucleic acid sequences, and the development of techniques that permit the monitoring of these sequences. Gene-cloning techniques and oligonucleotide synthesis now allow many nucleic acid sequences to be prepared in large quantities, resulting in the development of nucleotide sequence analysis and rapid probe assays for both bacterial and viral infections in fish (Gustafson et al. 1992; Pryde et al. 1993; León et al. 1994).

*X-ray Studies.* Radiographs are readily obtained in two ways. Firstly, the anaesthetized fish is briefly removed from the water. In this case the animal is placed onto a layer of film enclosed in waterproof material. Secondly, a restraint device such as a perspex box is used to hold the fish. However in this case there is some loss of detail as X-ray energy is lost through water. X-ray techniques have been used as tags, to estimate food intake, and to study skeletal structure (Bruno 1990; Kils et al. 1991).

### *Future Developments*

The wide-scale application of DNA-probe technology will depend on the development of routine nucleic acid sequence detection procedures which are accurate, sensitive, rapid and economical. The sensitivity of DNA probes can be greatly enhanced by using primer-directed enzymic amplification of target DNA (polymerase chain reaction [PCR]). By using PCR-based detection procedures, single organisms can be detected. The possibility of performing simultaneous screening in a single nucleic acid sample will be valuable in locating genetic markers in linkage analysis, detection of infectious agents or virulence factors, and also in surveying patterns of RNA expression in tumour tissue.

## APPROACHES TO DISEASE AND PARASITE MANAGEMENT

### *Management Of The Pathogen*

*Avoidance or Elimination of the Pathogen.*

*1) Use of pathogen-free water.* Open water supplies usually contain wild fish or invertebrates that may act as reservoirs of pathogens, placing fish at farms using that water at risk. However, some water supplies can be used that are free of potential infection reservoirs. These are mainly ground water supplies like natural springs or artesian wells. Some surface water bodies may be inaccessible to migratory salmonids, e.g., some mountain lakes or lochs, or dammed reservoirs, and these can be useful sources of pathogen-free water. Usually limited amounts of such water are available but they are invaluable water sources for hatcheries where fry can be reared to a more resistant age before being placed into exposed sites. This is particularly useful in controlling losses from certain virus diseases like IPN where first-feeding fry are highly susceptible but older fry are more resistant.

As an alternative to natural sources of pathogen-free water, water supplies can be disinfected by ultraviolet (UV) light treatment. This method has limitations as only relatively small volumes of water can be treated, although some new units are capable of treating 20,000 $m^3/h$. Some viral pathogens, e.g., IPNV, are quite resistant to inactivation by UV, but the method is employed in salmonid hatcheries to control certain bacterial pathogens such as *Flexibacter*, *Aeromonas* and *Renibacterium*. Other methods are forms of water filtration. Sand filters may be useful to avoid parasitic diseases and possibly bacteria. A sophisticated and expensive method is a series of filter plates with micropores to remove particles as small as bacteria.

Ozone treatment units have recently been introduced in salmon smolt units in Norway. They have capacities to disinfect 2,500 l/min. Retention tanks are required to retain treated water until the ozone converts to oxygen before supplying the fish. This method has the bonus of enriching the oxygen in the water.

*2) Reduction of vertical disease transfer.* Several pathogens can be present in the sex products of infected brood fish and persist either in or on the eggs to infect the next generation of fry. Pathogens that can persist within the ova are IPNV, IHNV and *R. salmoninarum*. Others which may persist on the ova and infect fry on hatching include *A. salmonicida* and VHSV.

Disinfection of eyed ova to remove surface-borne pathogens is relatively simple, safe and effective. Buffered iodophores are usually used at the eyed stage of ova. However, disinfection procedures to remove pathogens which can be inside the egg are not so straightforward. Eggs and milt have been treated with iodophore before and during fertilisation to prevent the IPNV entering the micropyle with the sperm at the moment of fertilisation. While this procedure is considered to decrease the chances of vertical transmission, it is not completely effective and toxicity of the disinfectant to the gametes can also result in considerable egg mortality or abnormal development of the embryos. Egg-disinfection procedures are not effective when the pathogen is already inside the egg before spawning, as may occur with *R. salmoninarum*.

A relatively successful strategy for significantly reducing, but not eliminating, vertical transmission of *R. salmoninarum* has been to inject female broodfish with certain antibiotics, e.g., erythromycin, oxytetracycline or rifampicin, into the dorsal sinus, 2-3 weeks prior to spawning (Brown et al. 1990). At the alevin stage the percentage of infected individuals was reduced from about 90% in the controls to 20% by this treatment.

The most effective means of controlling vertical transfer of infectious agents is to test for pathogens in brood stock at the time of stripping. Ovarian fluid, milt blood and kidney samples can be collected from live fish during or immediately after spawning. These should be tested by laboratory techniques for the suspect pathogen. While this is being done, the batches of eggs are incubated in separate lots in the hatchery and their parents identified by marking. If any of the individual parents are found to be positive, the eggs and parents should be destroyed. Such practice has been applied in Scotland with respect to IPN and BKD and the relatively confined spread of these diseases in this country can be attributed to this policy (*see* Chapter 4).

*3) Eradication of infected stock.* Obviously this is commercially a drastic step to take, especially when state compensation may not be forthcoming even when state regulations might require eradication of stock. It is practised only rarely and when potentially calamitous circumstances may result; for instance, the introduction of an important exotic pathogen into an area previously free of that disease. The European Community directives may require compulsory slaughter of stock if infectious salmon anaemia (ISA) is diagnosed and also for IHN and VHS in zones which are approved to be free of these diseases. Emergency cases where such measures have been practised in the past, as with the introduction of furunculosis into Norwegian salmon sea-cage farms and the first IHN outbreaks in rainbow trout in France and Italy, were not, unfortunately, successful in eradicating the pathogen, which spread to other farms and rapidly became endemic.

*4) Certification programs.* Many countries employ regulations to prevent the movement of eggs or fish infected with certain so-called "notifiable" diseases from an infected to a non-infected site. These policies are designed to limit the spread of the disease. When these facilities are available it is possible to have frequent tests performed and stocks certified to be free of certain diseases. Such certification is required for international trade in live fish and eggs, but within a country it is widely practised voluntarily as stocks certified to be "disease-free" command premium prices.

*5) Facility design.* Farms and husbandry practice can be designed in such a way as to avoid the introduction of disease agents and to restrict their spread within a farm in various ways.

*a) Isolate the hatchery*: If a pathogen-free water supply can be used to supply a hatchery, it is important not to introduce infections from surface water supplying other parts of the farm. The hatchery should then stand apart and strict hygiene standards applied to equipment and personnel entering the hatchery.

*b) Hygiene practice*: Limiting the horizontal spread of disease agents on a farm should be practised at all times. Such practices include having hand nets for each tank and disinfecting the net after each use, and the separation of the rearing facilities into discrete units. Disinfection foot-baths and restricted movement of personnel between buildings, protective clothing and other equipment are important management practices. Prompt removal of dead and moribund fish is essential as large numbers of pathogens are shed into the water from such fish. In small tanks this is easy to perform with hand nets, but in large sea-cages lifting the nets is very stressful to the fish and employment of divers is expensive. Recent modifications to the construction of cages fitted with "dead-socks" have proven highly successful. These nets are constructed with a funnel shaped extension (a holster) to the bottom of the cage in which rests a net lining or "sock". Dead fish fall into the sock and with a pulley system, the sock can be hoisted out of the water for dead fish to be removed and then relocated into the holster (Figure 1). By this method dead fish can be removed daily with little effort to farm personnel and little stress on the fish; this can be automated in larger cages. Dead fish should be disposed of properly, away from the farm and any watershed, in limed pits, silage plants or by composting.

*c) "All-in, all-out" practice, alternatively referred to fallowing* [*see also* d)]: If a disease is introduced onto a site, it is important to restrict horizontal spread especially to different year classes of fish. Hence, before a new year class of fish is introduced to a part of the farm, all tanks and equipment should be thoroughly cleaned and disinfected. In sea-cage sites it is a useful technique to physically separate year classes to break the infection cycle.

*d) Fallowing and management agreements:* Most obligate pathogens do not survive for long periods outside their hosts and allowing a site to fallow for a period may eliminate or drastically reduce the pathogen load. The practice of fallowing has proved very effective in controlling furunculosis and salmon lice with the attendant improvement in stock growth and survival. This approach has also decreased the need to employ expensive medicinal treatments with their problems associated with tissue residues, environmental effects and resistance to antibiotics. In

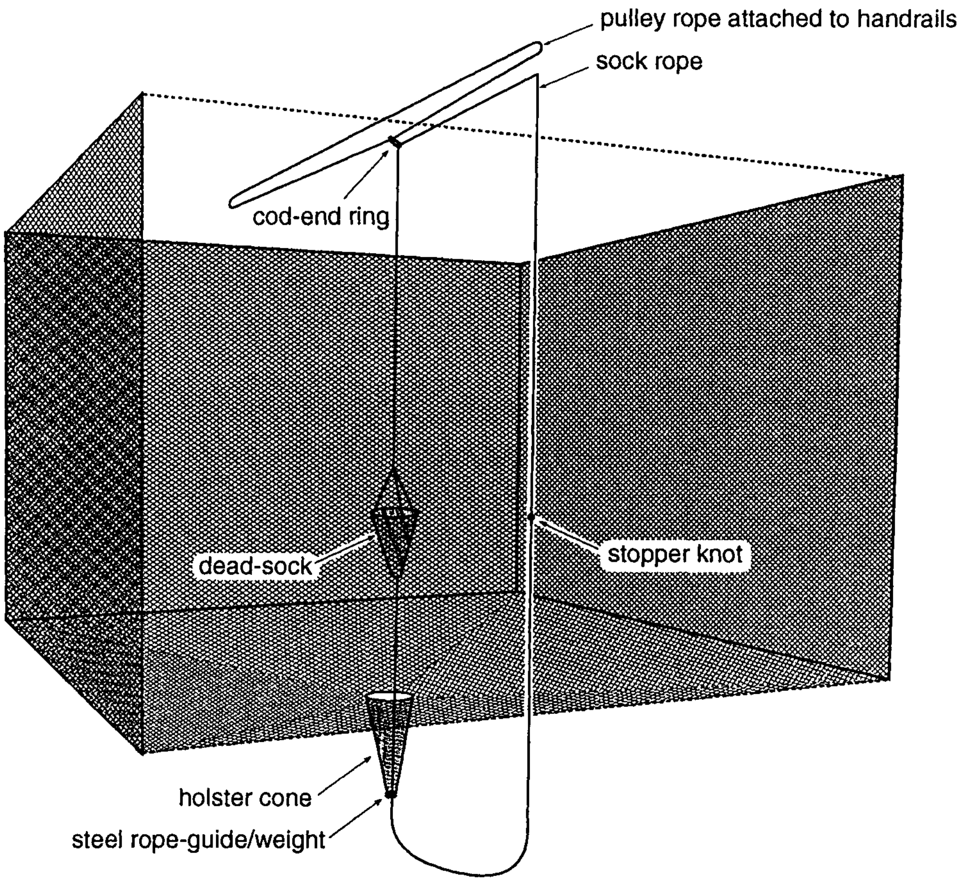

FIGURE 1. Diagram showing the placement of a "dead sock" in a sea cage. (For further information contact: Scottish Salmon Growers Association, Drummond House, Scott Street, Perth, PH1 5EJ, Scotland)

seawater Atlantic salmon farming, this principle has led to companies sharing a sealoch infected with furunculosis to arrange for simultaneous harvesting of stock and to allow several weeks to pass before introducing new smolts certified free of furunculosis. In this way annual serious outbreaks of furunculosis have been eliminated. Furthermore, serious outbreaks of sea lice on salmon have also been reduced in the first year in seawater. The lice population does build up again in the second year but to levels requiring less treatment. This practice would seem to be set for application on a much more widespread scale in the future.

### *Management of The Host: Stress Management*

#### *The Role of Stress in Fish Disease.*

*1) The stress concept.* Stress is a response in an organism to a change in the environment. This response is functional when the organism's relationship to the environment is brought back to a steady-state of "normality". Thus the stress response allows the animal to adapt to a changing environment. However, as with all responses, it is metabolically costly and can only be sustained for a certain period of time. If normality is not achieved within this period, metabolic processes are stretched and begin to breakdown so that the health of the organism is compromised. The changes that occur in the stress response are termed the General Adaptation Syndrome (GAS). They are of a physiological and biochemical nature and take place in three phases:

*a) Alarm reaction*: when metabolic and physiological resources are mobilized.

*b) Stage of resistance*: when the organism's physiological state returns to normal following the establishment of a sustainable relationship with the environment.

*c) Stage of exhaustion*: when the responses have not achieved a state of physiological homeostasis and bodily functions become compromised.

The changes occurring during GAS are mediated by hormonal and nervous reactions, principally the release of adrenocorticotropic hormone (ACTH) from the pituitary, which causes a release of corticosteroid hormone, mainly cortisol, from the interrenal tissue in salmonids. Nervous responses in the alarm reaction also stimulate release of catecholamines (e.g., adrenaline) from chromaffin tissue, also located in the anterior kidney of salmonids (*see* Barton and Iwama 1991).

*2) Key stressors in the aquaculture environment.*

*a) Water quality*: Salmonids have high oxygen requirements and circumstances that reduce the available oxygen levels in water such as high temperature or microbial blooms, induce respiratory stress.

*b) Crowding*: Crowding affects water quality by reducing oxygen and increasing toxic waste products such as ammonia in the water. Fin nipping and aggressive encounters are common stressors in intensive aquaculture.

*c) Handling*: Various types of handling (netting, grading, marking, transport) are forms of physical stressors (*see* Chapter 12).

*d) Disturbance*: Farm workers or predators such as herons or seals disturbing fish can cause stress in "nervous" species like Atlantic salmon.

*e) Nutrition*: Proper nutrition is important for good health and problems have been encountered with certain vitamin deficiencies, e.g., vitamins E and C, especially when food is stored badly (*see* Chapter 10).

*f) Hierarchical*: Effects may develop at certain population densities or between fish of different sizes leading to aggressive encounters.

*3) Consequences of stress.* The hormonal changes occurring during acute stress mediate physiological changes primarily in the respiratory, circulatory and osmoregulatory systems. The most immediate consequences of stress (e.g., from netting, grading, transport) are on the respiratory system. For instance, the oxygen consumption of farmed rainbow trout is still elevated by 50% one day after fish have been graded. Problems of oxygen demand are exacerbated at high temperature when the fish's requirement for metabolism is increased while the oxygen-carrying capacity of the water is decreased. In extreme circumstances, for example, transport at high stocking density, respiratory stress can be directly responsible for mortality. Even when dissolved oxygen levels are adequate, at high stocking density toxic metabolic products, e.g., ammonia and carbon dioxide, can accumulate and contribute to respiratory stress (*see* Chapter 12).

A further consequence of the respiratory adjustments of stressed fish may lead to salt or ion imbalance. Salts are lost by fish in fresh water while there is an influx of salts in seawater. This imbalance places pressure on the osmoregulatory systems which can result in mortality due to osmoregulatory failure.

Changes also occur in the defence mechanisms during stress (Barton and Iwama 1991). Chronic stressful conditions can result in a breakdown in the defence mechanisms of fish and an increased susceptibility to infectious agents, many of which would not normally cause disease in salmonids, e.g., *Aeromonas hydrophila* (*see* Pickering 1993). Stress can also decrease the effectiveness of a vaccination program since the specific immune response may be suppressed by stressful circumstances. Thus, it is important to minimise stress before, during and after the vaccination procedure.

*4) Reducing stress in the aquaculture environment.* It is not possible to eliminate all procedures known to induce stress in cultured salmonids as many are integral components of fish farming, e.g., netting, grading, transport. However, it is possible to minimise the effects of these stressors and others, such as overcrowding and variable water quality, can be avoided. Farmers are familiar with the guidelines on stocking densities, water flow, and feeding rates for salmonid cultivation. Severe consequences from stress could follow from exceeding those limits. In cases where stressors are unavoidable, certain strategies can be employed by the farmer to minimise the effects of stress (*see also* Chapter 12).

*a) Permit recovery*: Generally the duration of the recovery period is proportional to the duration of the stressor. Thus, reducing the duration of netting, grading or transport will result in recovery in a shorter time. Nevertheless, it should be noted that recovery can take an appreciable time. For example, some of the secondary effects of a 30-second handling stress may last for several days and recovery periods of two weeks are recommended for salmonids (Pickering 1993).

*b) Avoid multiple stressors*: The effects of multiple stressors can be additive or even synergistic; e.g., the cortisol response in acid-exposed rainbow trout to acute handling stress was more than twice that of unexposed fish. Sudden temperature changes should be avoided during or after transport. Repeated handling stresses three hours apart can have a significant cumulative effect on the fish's stress responses (Barton et al. 1986).

*c) Avoid stressors at high temperatures*: Stress-induced mortality increases at high water temperature and so it is safer to carry out netting, grading and transport at low water temperatures, although sudden temperature change itself can be stressful.

*d) Withdraw food prior to handling*: Following a meal the oxygen requirement of salmonids increases to provide the energy demands of digestion. Withdrawal of food two or three days prior to handling will therefore minimise respiratory stress. It also avoids fouling the water with faecal material and regurgitated food.

*e) Reduce osmotic stress*: In fresh water, the use of dilute salt solutions during transport has been shown to limit the loss of ions from the fish and significantly reduce stress-associated mortality.

*f) Use of anaesthesia*: Although anaesthesia itself can disturb the fish's physiology, light anaesthesia can have a calming effect on fish during handling exercises and reduce the stress resulting from them.

*g) Mimic natural environment*: Overhead cover is an important component of the young salmon's environment, particularly during winter months. Provision of a floating overhead cover doubled the growth rate of Atlantic salmon parr, increased the proportion of potential S1 smolts, minimized haematological signs of stress, and halved the mortality rate of potential S2 smolts during the first winter (Pickering 1993).

*h) Future development — selective breeding*: It is now apparent that the magnitude of the stress response is at least a partly heritable characteristic in salmonids (*see* Pickering 1993) and programs have begun to select brood stock that have a low cortisol response to handling stress. In this way the process of domestication can be accelerated to produce stocks more suitable to tolerating aquaculture stressors with resultant benefits of increased health, survival, and productivity of fish raised for market.

### *Vaccination*

Vaccination plays an important role in salmonid culture but it is not a panacea. In all forms of intensive agriculture where many individuals of a single species are reared in densely populated circumstances, infectious agents can build up rapidly if the general health status of the population is low. If the animals' defence systems are compromised by stress, the protection afforded by vaccination can be overwhelmed. Thus, vaccination should be used as part of a broad-based husbandry strategy emphasising hygiene to minimise exposure to pathogens, and also minimise the stress induced by certain integral aspects of salmonid culture (as discussed above). During the last 15 years, vaccination has played a key role in controlling ERM in rainbow trout culture, and it is widely held that salmon farming in brackish and marine environments in many parts of the world has been made possible by the existence of vaccines against vibriosis. For detailed reviews of fish vaccination see Ellis (1988), Newman (1993) and Leong and Fryer (1993).

*Historical Perspectives*. The first attempts to vaccinate fish were in the 1930s to aid control of furunculosis in North American salmonid hatcheries (Duff 1942). With the advent of antibiotics in the 1950s, interest in fish vaccination waned. Salmon farming on a large commercial scale began in the 1960s at a time when the major bacterial diseases, such as vibriosis, furunculosis and ERM, were successfully controlled by antimicrobial chemotherapeutants. However, as the industry grew rapidly in size during the 1970s and early 1980s, these bacterial pathogens developed resistance to many antibiotics and chemotherapy became more difficult and expensive. Furthermore, there are legal constraints imposed on the use of chemicals in order to control potential hazards to human health, to the environment, from persistent residues in the fish flesh and from handling. The compounded problems with antibiotic resistance has highlighted vaccination as a highly desirable method of disease control in aquaculture.

*Current Status of Salmonid Vaccination*. The vaccines currently used commercially in salmonid culture are all against bacterial diseases (Table 7). The main reason for this is production costs. Obviously, the cost per dose of vaccine must be very small. Preparation of viral vaccines involving growing the virus in tissue culture is very

TABLE 7

Current status of salmonid vaccination.

| Commercial vaccines | | Experimental vaccines |
|---|---|---|
| **Disease** | **Bacterial gent** | **Viral disease** |
| Vibriosis | *Vibrio anguillarum*<br>*Vibrio ordalii* | IHN<br>VHS |
| Cold water vibriosis | *Vibrio salmonicida* | IPN |
| Enteric Redmouth (ERM) | *Yersinia ruckeri* | |
| Furunculosis | *Aeromonas salmonicida* | |

expensive. On the other hand, it is inexpensive to culture most bacteria in large fermenters and then chemically inactivate the toxins and bacterial cells (usually with formalin treatment). Such simple crude vaccines, termed bacterins, have proved highly effective in protecting against vibriosis, cold-water vibriosis and enteric redmouth disease. However, furunculosis bacterins have not been so effective. A great deal of research has been conducted on furunculosis in attempts to elucidate the pathogenic mechanisms and virulence factors of *Aeromonas salmonicida*, in the expectation that such knowledge would lead to the identification of antigens which could stimulate a protective immune response. Recently, certain cell-surface antigens of *A. salmonicida*, which are only expressed when the bacterium is grown under iron-restricted conditions and termed iron-regulated outer membrane proteins (IROMPs), have been shown to induce protective bactericidal antibodies (Hirst and Ellis 1994). A new generation of furunculosis vaccines are now commercially available based on growing the bacterium under iron-restricted conditions.

While most pathogenic fish bacteria grow well in nutrient media, *Renibacterium salmoninarum* is extremely fastidious and slow growing. Furthermore, crude bacterin preparations have failed to induce protection and much more research is required to identify potential protective antigens of this bacterium before vaccination against bacterial kidney disease becomes feasible (Munro and Bruno 1988).

Experimental vaccines based on injection of inactivated wild-type IHNV, VHSV and IPNV have been shown to induce protection in fish. However, in some cases, e.g., IPN, a large dose of virus is required. The commercial constraints on producing these viral vaccines for the salmonid farming industry concerns the very high costs of culturing the viruses in tissue culture. Hence, in recent years molecular biological approaches have been taken to reduce the costs of producing viral antigens which could be used in developing vaccines for fish (see Future Developments below).

Much the same constraints of production costs that apply to viral vaccines also apply to parasite vaccines, though some success has been made in culturing a free-living protozoan, *Tetrahymena* sp., which has cross-reacting antigens with *Ichthyophthirius* and which induce protection against *Ichthyophthirius* (Goven et al. 1981). However, other workers have been unable to confirm this (Clark et al. 1988; Houghton et al. 1992). In order to develop vaccines against large metazoan parasites of salmonids like sea lice, molecular biological approaches to antigen production is essential (*see* below).

*Methods of Vaccination.* The different circumstances that apply to the farming of different salmonid species as well as the needs of vaccinating fish of different sizes have given rise to a number of different methods of administering vaccines. The basic methodologies are injection, immersion and incorporation in the feed, i.e., oral administration. The relative advantages, disadvantages and applications are summarized in Table 8 and methods of administration in Table 9.

*1) Injection.* It is essential for injection vaccination that fish are anaesthetized, not merely to facilitate handling but to avoid excessive stress. The vaccine is usually injected using multidose syringes. Fish are injected immediately anterio-laterally to the pelvic fins, with the syringe held at an angle of about 25° to the body surface so that the inoculum enters the peritoneal cavity with a minimum risk of damaging the underlying organs. Commercially available vaccines are mostly formulated for a dose rate of 0.1 ml independently of fish size.

The rate of injection achieved depends on the individual weight of the fish and the skill of the team. Over a sustained period an experienced handler will inject 1,000 to 1,200 fish per hour when conditions are favourable and the fish are in excess of 20 g. The rate achieved rarely falls below 600-700 per hour even under poor conditions with inexperienced workers.

Injection is an advantageous mode of administering vaccines from the point of view of both the protection parameters and economics (*see* below). However, it is labour intensive and relatively slow, and becomes impractical when fish are lss than 15 g. It is, therefore, predominantly used when Atlantic salmon are transferred as smolts to seawater, or when large trout are transferred to seawater for ongrowing to smoking size or for potential brood stock. Semi-automated machines have recently become available and can inject up to 4,000 fish per hour.

*2) Immersion methods.* All immersion methods in routine use expose the outer surface of the fish directly to diluted vaccine (direct immersion) relying on uptake, predominantly by the gills and to some extent by imbibition, to take place passively. There are two variations of direct immersion; dip and bath vaccination.

TABLE 8

A summary of advantages and limitations of injection, immersion and oral vaccination methods.

| Method | Advantages | Limitations |
|---|---|---|
| Injection | Most potent immunisation route. Allows use of adjuvants. Most cost-effective method for large fish. Semi-automated machines available. | Labour intensive. Stressful (anaesthesia; handling). Fish must be >15 g. Hazardous to injection personnel. |
| Immersion | Allows mass vaccination of small (<5 g) fish. Most cost-effective method for fish <10 g. Bath: not stressful. | Dip: handling stress. Potency not as high as injection route. Not economic for large fish. Adjuvant delivery problematical. |
| Oral | Non-stressful. Allows mass vaccination of fish any size. No extra labour or machinery costs. | Variable protection levels, lower than other methods, probably best used to boost vaccinate. |

*a) Dip vaccination.* A small tank containing vaccine is placed in close proximity to the holding tanks. The volume of the vaccine will depend on the manufacturer's instructions, but most commonly vaccine is diluted one part vaccine to nine of clean water at the same temperature as the fish. Each litre of undiluted vaccine contains sufficient antigen for 100 kg of fish. Therefore for each 100,000 fish at 4.5 g (approximately 100 per lb) being vaccinated, 4.5 l of vaccine will be required to give a working volume of 45 l when diluted. The water level of the tanks holding the fish is lowered to facilitate catching fish. Small netfulls of fish are taken, drained (to avoid diluting the vaccine) and still retained in the net, and immersed in the vaccine from 20 seconds to one minute according to the specific manufacturer's recommendation. The net of fish is then drained of vaccine and the fish are returned to a fresh holding tank. Typical recommendations of density of fish in the vaccine are 450 g of fish per litre of diluted vaccine.

*b) Bath vaccination.* The requirement for vaccination without netting or handling has led to the increasing use of the "bathing" methods, in which vaccine is added directly to the holding tank. The simplicity of this administration, however, is counter-balanced by the much greater amount of vaccine required.

TABLE 9

Methods of administering vaccines to fish.

| Route | Vaccine dilution | Exposure time |
|---|---|---|
| Immersion dip | 1:3, 1:10, 1:100 | 5-30 seconds |
| Immersion bath | 1:500, 1:5000 | One hour or longer |
| Innoculation | Undiluted (0.1 ml) | Direct injection |
| Oral | In food | One week or longer |

Addition of one part in 10 to a holding tank would require an excessive volume of vaccine. However, a more dilute solution requires the exposure time to be increased if the amount of vaccine taken up is to be sufficient to ensure long-lasting immunity.

The "standard" procedure for bath vaccination was developed in Norway and is widely used for the administration of vaccine to Atlantic salmon at the pre-smolt stage 4-8 weeks before transfer to seawater. The water level of the holding tank is lowered to the minimum level in which it is judged the weight of fish in the tank can safely be held, with oxygenation, for one hour. Vaccine is added to this volume to achieve a dilution of one part in 500. After one hour the water flow is restored and the tank volume brought back to normal with the vaccine slowly diluting away. On average 40-60 kg of fish may be vaccinated per litre of vaccine.

*3) Oral vaccination.* For many fish farmers, merely adding antigen to the food for a suitable time course is compellingly simple and attractive. The earliest attempts at fish vaccination used oral administration, and much research has been devoted toward this end for all of the bacterial vaccines currently in use. It is widely held that protection achieved by this method takes longer to achieve and is poorer than with the other methods. However, careful scrutiny of the literature shows that this is not certain and that when sufficient antigen mass is administered, protection may be acceptably high. Much effort is now being devoted to oral delivery of vaccines to fish, particularly aimed at protecting the vaccine against digestion in the stomach and anterior intestine and to improving the uptake of the vaccine in the hind-gut. It is known that this region is immunologically responsive in salmonids and given that the vaccine can reach this zone intact, the potency of oral vaccination might be markedly improved.

*When to Vaccinate.* Ideally, vaccination should fit into the husbandry strategies employed on any particular farm, but two factors that change with time are of major and general importance, the size of the fish and the water temperature.

*1) Size of fish.* The immune response of fish takes some time to mature after hatching. Size, not age, is the significant factor. In salmonids a high level of immunity is achieved only in fish weighing more than 1 g. However, the immunity induced at this size appears to decline after about three months, whereas fish vaccinated at 4 g retain immunity at a higher level for at least a year.

*2) Temperature.* Most aspects of the physiology of fish, including the rate of acquisition of immunity, are affected by temperature. In general, protective immunity develops more slowly as the temperature falls below the optimum for any particular species. However, most vaccine manufacturers indicate a point, usually 5-6°C, below which vaccination is not recommended, and, where the option exists, as high a temperature as possible, up to the optimum for the species, should be used.

*Economics of Fish Vaccination.* Whether a fish farmer will use a commercial vaccine is basically an economic decision and a cost-benefit analysis has to be carried out. It must be realized that no vaccine for animal or human is 100% effective. Some individuals in any population will not be effectively protected for prolonged periods and some diseases will still occur, making other forms of disease control, e.g., antibiotic therapy, necessary.

However, a cost-effective vaccine will not only reduce economic losses through reduced mortalities, but should also alleviate decreased growth rates due to infection, and reduce the need for chemotherapy. Thus, the cost-benefit analysis needs to be based upon certain expectations of how well a vaccine might perform given certain conditions under which the farmer is considering its use. Such expectations need to be based upon the results of large field trials which are appropriate to the type of farm in question.

To date most available data come from the use of ERM vaccines in rainbow trout farms. A detailed economic analysis of these data has been made by Horne and Robertson (1987). For the basis of calculation it is assumed that the cost of ERM and vibriosis vaccines is about £100 (UK) per litre. The cost per fish depends upon the method of delivery and the size of the fish. The injectable vaccine dose is 0.1 ml per fish irrespective of the fish's size (i.e., 1p per fish), while the dose of the immersion vaccine is calculated at 1 litre per 100 kg fish, i.e., 1p for a 10 g fish, 10p for a 100 g fish. Thus the most cost-effective means for vaccinating fish below 10 g is the immersion method, whereas for fish progressively larger than 10 g the injection approach becomes more cost-effective, though in practice it is not realistic to inject fish under 15 g.

In an economic analysis of immersion vaccination of 4.5 g rainbow trout against ERM (fish being reared to a mean individual weight of 250 g) based upon available field data, Horne and Robertson (1987) calculated that if vaccinating fish resulted in 1% more fish reaching market the cost of the vaccine would be recovered. Moreover, increased survival of vaccinated fish is not the only economic benefit. The field data on ERM vaccinated rainbow trout indicated that savings in the cost of medicated feed of up to 70% and also increases in food conversion ratios may be expected.

### *Future Developments*

As mentioned above, with the exception of *R. salmoninarum*, effective experimental vaccines against most of the economically important bacterial and viral diseases of salmonids have been produced. But the problem with extremely high production costs for the viral vaccines has precluded them from commercial development. In recent years, molecular biological techniques have been employed to produce recombinant vaccines to overcome this problem. Essentially, this approach involves extracting the genetic material of the pathogen, cutting it into fragments and inserting (recombining) them into a bacterium (usually *Escherichia coli*). These gene fragments can then be induced to express the protein for which they code by growing the bacterium in conventional fermenters. High level expression of the cloned protein can be achieved very cheaply. The molecular techniques used to select and clone the desired antigens are very costly and the funding necessary to carry out the fundamental work is beyond affordability by fish vaccine companies. Thus, support from the industry in general and by government-sponsored research councils is essential to the success of this approach. However, once the genes for the protective antigens have been selected, cloned and expressed, the final production cost of a commercial vaccine is very low.

Considerable advances have been made in developing vaccines to IHN and VHS in recent years using these techniques (Leong and Fryer 1993; Lorenzen et al. 1993) and similar programs are in progress with vaccines for IPN, salmon louse and BKD. In the near future the commercial availability of some of these vaccines can be expected.

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## Chapter 14

# OCEAN RANCHING: AN ASSESSMENT

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## INTRODUCTION

Only within the last two decades has the phrase "sea ranching" or "ocean ranching" come into common usage. The idea revolves around important principles of stewardship, including husbandry and managed use of renewable marine resources. The notion of "ranching" marine resources in open ocean pastures is not restricted to salmonids (Koganezawa 1991; Isaksson 1989) and the term likely came from and can relate to the practice of managing livestock on open rangeland.

Original concepts for ranching anadromous salmonids grew out of the first realization these fishes homed to natal waters after an ocean odyssey, and that somehow numbers were related to reproductive success of annual spawning runs. Even before modern fisheries science, aboriginal cultures developed notions of user-rights systems that included a sense of husbanding specific runs of salmon for future benefit (Rogers 1960).

### *What is Ocean Ranching?*

Ranching of salmonids could include any anthropogenic activity having an effect on abundance of these resources. Unfortunately, most interaction between man and salmonids historically has not been particularly beneficial for the fish (Netboy 1968; 1980). In a broader context, concepts of ranching salmonids should logically include harvest-management practices and fisheries that target regional or specific stock groups of fish along with watershed and land-use practices. Production lost due to habitat deterioration, urbanization, and industrialization, concomitant with efforts to mitigate these losses, are all part of the legacy of how we "ranch" or manage these resources.

One of the more successful "ranching" efforts, for example, was the development of fish passages at Hells Gate on the Fraser River in British Columbia, Canada. This feat, merging engineering and biology so that migrating adults could bypass velocity barriers created by massive landslides, reclaimed the productive potential of the upper Fraser watershed for anadromous runs of sockeye (*Oncorhynchus nerka*), and pink (*O. gorbuscha*) salmon (Roos 1991). Management for optimizing escapements of naturally spawning populations to maximize subsequent yield, recognizably a difficult task (Larkin 1979), integrated with effective, biologically sound harvest strategies for both hatchery and, where appropriate, wild stocks is a logical goal for ocean ranching.

Although conceptually appropriate in a broader context, contemporary perceptions of ocean ranching normally apply only to a limited subset of this holistic view. This chapter, then, will focus on the more restricted view of ocean ranching as the artificial rearing of salmonids released as juveniles to migrate and grow in marine waters and return as adults either to the release site or to become available for harvest in particular areas (McNeil and Bailey 1975; Thorpe 1980; Allee 1988; Isaksson 1988; Kaeriyama 1989*a*).

The intent of the chapter is to review why and how ocean ranching is practiced in selected areas, examine assumptions associated with the planning for ranching, and consider performance measures of some ranching programs.

## *Reasons For Ocean Ranching*

Salmonid ranching in different areas varies widely, both in its focus and complexity of application. Ranching programs may encompass diverse objectives, goals and projected benefits, including: (1) restoration of depressed natural runs through intermittent enhancement and supplementation of wild fish stocks with hatchery-run fish; (2) permanent hatchery-based populations as mitigation for irrevocable habitat losses; (3) creating new runs in areas where none occurred before; (4) hatchery runs for scientific research or educational purposes; and (5) conservation measures to save threatened or endangered anadromous stocks from extinction or to preserve unique genetic or behaviour features of populations.

In some countries, for example, Iceland (Isaksson 1989) and Norway (Tor Heggberget, pers. comm.), ranching is considered somewhat distinct from enhancement. Although both may involve culture and release of juveniles for anadromous returns, enhancement in these instances is done primarily to aid in rebuilding depressed wild runs. Ranching, under these conditions, is reserved for closely managed fisheries dependent solely on hatchery-based runs of fish.

In North America, ranching often is considered more synonymously with enhancement (Allee 1988) or perhaps as only one component of a wider suite of enhancement activities. Other viewpoints of salmonid ranching involve concepts of public resource development and entrepreneurial principles of private commercial enterprise. Different views on ocean ranching arise from divergent societal and political backgrounds, including unique historical precedence and traditional uses of these resources.

Salmonid ranching may involve specific interest groups of resource users, for example, commercial, recreational or subsistence fishermen that might be restricted to certain types of gear for harvesting fish in specified areas. A key feature of private ranching is proprietary ownership of some portion of the returning adults for commercial profit. Assuming there is some level of biological success, ranching programs may provide secondary economic benefits to communities or regions in addition to improving fisheries.

## RANCHING PROGRAMS IN DIFFERENT AREAS

The following section highlights some of the diversity in ocean ranching as currently practiced in different areas. It is not intended be comprehensive; included are three programs focused on Atlantic salmon (*Salmo salar*) and four on Pacific salmon *Oncorhynchus* spp. (Table 1).

### *Iceland*

Atlantic salmon resources in Iceland involve two distinctive factors unique from other countries. First, no salmon fisheries are permitted in coastal Icelandic waters and returning adults can not be harvested until they migrate into rivers. Second, rivers and fishing rights in them are not a common property resource but are proprietary property of private land owners (Isaksson 1980, 1988, 1989; Scarnecchia 1989). Traditional commercial salmon fishing in Iceland, based on gill nets in streams, only developed to a minimal extent (Isaksson 1980). What did develop in Iceland is a famous and highly commercialized recreational sport fishery in local rivers based on the private ownership of these resources. Private owners of streams and salmon runs in Iceland frequently are successful in opposing hydroelectric development and other habitat-loss developments that would diminish these resources (Isaksson 1988).

Without a coastal salmon fishery and with a significant number of non-anadromous streams (Scarnecchia 1989), Iceland appears to have near optimal conditions for development of private ocean ranching. Isaksson (1980) reviewed these features and reported on initial developments of ranching in Iceland concentrated mostly in the southwest region of the country. His report included initial results of ranching research

TABLE 1

Some general features of salmonid ranching programs as practiced by different countries and regions.

| Country or region | Species (principal) | Species (other) | Approximate annual releases of juveniles in recent years (× 1000) | Approximate number of adult returns in recent years (millions) |
|---|---|---|---|---|
| Iceland | Atlantic salmon | | 6,000 | ? |
| Norway | Atlantic salmon | Arctic charr | 1,000 | ? |
| Sweden | Baltic salmon | | 5,000 | 0.4-0.8 (?) |
| Japan | Chum salmon | Pink salmon<br>Masu salmon<br>Sockeye salmon | 2,000,000 | 40-50 |
| British Columbia | Chinook salmon<br>Coho salmon<br>Sockeye salmon | Chum salmon<br>Pink salmon<br>Steelhead trout<br>Cutthroat trout | 650,000 | 7-8 |
| Oregon and Washington | Chinook salmon<br>Coho salmon<br>Steelhead trout | Chum salmon<br>Pink salmon<br>Cutthroat trout<br>Sockeye salmon | 500,000 | 2-3 (?) |
| Alaska | Pink salmon<br>Chum salmon<br>Sockeye salmon | Coho salmon<br>Chinook salmon<br>Steelhead trout | 1,200,000 | 20-40 |

at the Kollafjordur Experimental Station and an overview of probable oceanic migration patterns of Icelandic salmon.

In 1985 ranching of Atlantic salmon in Iceland involved the release of about 0.5 million smolts at 13 release facilities (Isaksson 1989). At this time another 2 million smolts were going into the newly developing sea-cage salmon farming operations. By 1989 releases of ranched smolts in Iceland had increased to 6 million, with roughly 2 million going into sea-cage rearing and 2 million into land-based rearing tanks (Gudjonsson 1991). Development of land-based rearing tanks grew out of frequent accidents with sea cages during storms and subsequent escapes of farmed fish. At maturity escapees from sea cages, especially those located near salmon rivers, were entering Icelandic rivers raising concerns for the long-term well being, especially genetic integrity, of wild stocks.

### *Norway*

In Norway ranching of Atlantic salmon is conducted on a small-scale, mostly experimental basis. By comparison, Norway is the leading producer of farmed Atlantic salmon reaching a production level of around 150,000 tons (t) in recent years (Folsom et al. 1992). Norway has about 500 streams that still support wild salmon (Heggberget et al. 1993) and production from these streams apparently is around 2000 t. Most releases of cultured salmon have been in streams to enhance depressed natural runs, especially where habitat losses have occurred, for example, from dams and impoundments (Hesthagen and Garnes 1986; Gunneröd et al. 1988).

Annual releases of hatchery-reared Atlantic salmon smolts for ranching in Norway apparently have increased from 288,000 in the mid-1980s( Gunneröd et al. 1988) to about 1 million by 1991 (Tor Heggeberget, pers. comm.). Most ranched Atlantic salmon smolts in Norway are marked before release. Norway also is conducting some ranching research with Arctic charr (*Salvelinus alpinus*).

### *Sweden*

At the turn of the century more than 7 million wild Atlantic (Baltic) salmon smolts were produced annually in Baltic Sea drainages from 60 to 70 salmon rivers (Eriksson and Eriksson 1993). Industrialized development, especially hydroelectric stations, has reduced the number of remaining streams accessible for natural spawning runs to about 20. Most of these are in Sweden and presently only about 0.5 million wild smolts are produced in the Baltic waters. Major smolt rearing programs for ranching of Baltic salmon were developed to mitigate losses of natural production. Many individual hatchery stocks are maintained from the original streams that were blocked by dams. Larsson (1980) listed 18 Swedish smolt rearing stations and nine or more rivers where hatchery-reared smolts are released.

In addition to releases of ranched smolts in rivers, a system of Swedish coastal and delayed sea releases of smolts from cages in the Baltic has proven successful in further mitigating losses of natural spawning (Eriksson 1989*a*, 1989*b*; Eriksson 1991; Eriksson and Eriksson 1991). The total annual smolt production of Baltic salmon is presently near historic levels; however, roughly 90% is of hatchery origin. Moreover, a highly efficient offshore mixed stock fishery in the main Baltic is based mostly on this successful ranching program of hatchery smolts.

### *Japan*

Both in the number of hatcheries and in the number of juveniles released annually, Japan operates the most extensive ranching program in the world. According to Dr. Kazunori Kawagoe, Director, Hokkaido Salmon Hatchery (pers. comm.), there are 150 production salmon hatcheries on Hokkaido including 31 major federal facilities operated by the Japan Fishery Agency (JFA), six hatcheries operated by the Hokkaido prefectural government and 113 hatcheries operated by private fishermen cooperatives. On Honshu there were 165 private production salmon hatcheries in 1986 operated mostly by fishermen cooperatives (Nasaka 1988). Although there are federal laboratories on Honshu that culture salmonids, none apparently make production releases for ocean ranching. At present, roughly 2 billion juvenile salmon are released annually in Japan for ocean ranching, approximately half from Hokkaido and half from Honshu (Kaeriyama and Urawa 1993).

All salmon hatcheries in Japan are under the oversight of JFA by virtue of the Aquatic Resources Conservation Act of 1951 (Nasaka 1989). Coastal commercial fisheries for ranched salmon on Hokkaido and Honshu are based on cooperative fishermen groups and villages that have exclusive fishing rights to nearby trapnet sites, weirs or seining sites at the mouths of rivers. According to Nasaka (1988) Japan's national salmon hatchery program provides for subsidies to operate private hatcheries, and trapnet fishermen who benefit directly from the program make financial contributions toward paying for hatchery operations.

Kobayashi (1980) reviewed the background of salmon culture in Japan along with details of modern ranching technology that is focused primarily on chum salmon (*Oncorhynchus keta*). According to Kaeriyama (1989*a*), chum salmon account for over 90% of the commercial catch of ranched salmon in that country, with pink salmon accounting for 8%, and masu salmon (*O. masou)* and sockeye salmon less than 1% each. Although there are no endemic runs of sockeye salmon on Honshu or Hokkaido, Japanese scientists have successfully conducted ocean ranching research with this species by developing small anadromous runs from freshwater kokanee (landlocked sockeye) salmon populations (Urawa 1991; Kaeriyama et al. 1992).

Effectiveness of salmon ranching in Japan was greatly improved, beginning in the mid-1960s, when most hatchery fry were fed for short periods in rearing ponds and vernal release times were adjusted to reduce initial marine mortalities. These innovations led to dramatic increases in rates of adult returns from an average of about 1% of fry released to an average of over 2% afterwards (Kobayashi 1980; Mayama 1983, 1985). Returns of ocean-ranched salmon in Japan in 1987 included 42.9 million chum salmon, 3.8 million pink salmon, 744 thousand masu salmon and 1 thousand sockeye salmon (Kaeriyama 1989*b*).

Relative success of ranching programs in different regions of Japan depend in large measure on the different marine waters juveniles migrate into and adults return from (Figure 1). Japanese ranching programs have been studied by North American scientists (Mathews and Senn 1975; Moberly and Lium 1977; Shepherd 1984) and many elements of this successful hatchery technology, especially regarding chum salmon, are now widely used in other countries. Mathews and Senn (1975) observed that most chum salmon production in Hokkaido was based on hatchery releases of juveniles with almost no natural production. Kobayashi (1980) indicated there are about 350 salmon rivers in Japan but most now have unsuitable environmental conditions for efficient natural spawning due to habitat losses.

### *British Columbia*

The early history of salmonid hatcheries in British Columbia, Canada, as reviewed by Childerhose and Trim (1979), indicates that all initial programs were discontinued by the late 1930s and early 1940s. By the late 1960s and early 1970s, however, renewed interest in ranching in the province found hatcheries or spawning channels at Big Qualicum River, Quinsam River, and Robertson Creek on Vancouver Island, and at Capilano River and Babine Lake on the mainland (Childerhose and Trim 1979; Sandercock 1982). Initial successes with these and other ranching activities led in 1977 to the development of a major new Salmon Enhancement Program (SEP) by joint agreement of federal and provincial governments.

SEP is a broad-based concept that includes specific biological, social and economic goals for the province. The primary goal is to stabilize and increase salmonid stocks. This in turn provides a means of achieving socio-economic goals including: increasing regional, provincial and national income; creating new employment opportunities; improving economic opportunities for native peoples; stimulating economic development in outlying communities; and increasing and improving recreational opportunities (DFO 1989*a*). The program now uses, in addition to traditional hatcheries, other techniques including artificial spawning channels and nutrient enrichment in lakes. While neither spawning channel or lake fertilization techniques normally involve direct "hands-on" fish culture, both can be successful in increasing the numbers of juveniles going to sea and of returning adult salmon.

British Columbia spawning channels are designed to provide high quality artificial spawning areas where naturally spawned fry can migrate to under-utilized rearing environments. These channels, designed mostly for pink or sockeye salmon, have been used both to mitigate for losses of natural spawning grounds and to create totally new spawning areas. One large program, the Babine Lake Development Project, operates three channels on two stream systems and produces over 1 million adult sockeye annually (West and Mason 1987).

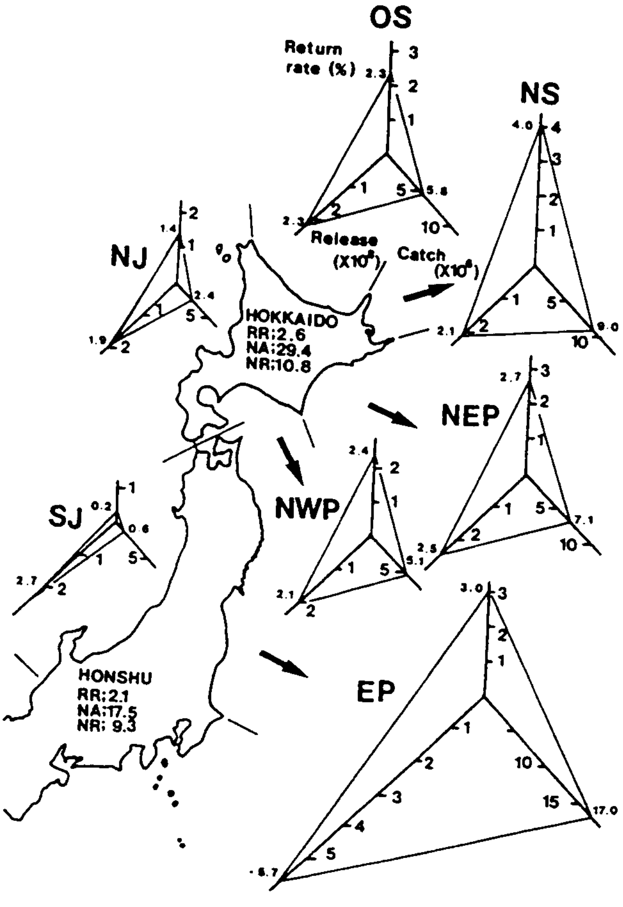

FIGURE 1. Average return rate (RR), numbers of adult returns (NA), and released juveniles (NR) for various areas of Japan in 1985-1987. OS, Okhotsk area; NS, Nemuro area; NEP, northeastern Pacific area; NWP, northwest Pacific area; NJ, northern Japan Sea area; EP, eastern Pacific area; and SJ, southern Pacific area. Return rates expressed as: NA/NR at age 4 from parent brood year (from Kaeriyama 1989*a*).

Lake fertilization projects, designed primarily to improve juvenile and, subsequently adult sockeye salmon production, increase primary productivity and appropriate food webs through nutrient enrichment (Stockner et al. 1980; Stockner 1987). Early on, the Great Central Lake fertilization program on Vancouver Island increased average sockeye salmon production to over 360,000 adults, seven times pre-fertilization levels (LeBrasseur et al. 1978).

Much ocean ranching in B.C. is focused on chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon. In 1977 when the SEP program began, there were already 11 chinook and coho salmon hatcheries in operation; in 1988 chinook salmon juveniles were released from 81 facilities and coho salmon juveniles from 218 facilities in B.C. (Cross et al. 1991). By contrast during the same release year, according to the SEP 1988-89 update report, sockeye, pink, and chum salmon were released from 16, 20 and 67 facilities, respectively (DFO 1989*b*).

Adult chinook salmon caught by fishermen from ocean ranching programs in B.C. during the period 1978-1989 ranged from 102.5 thousand to 266.7 thousand fish per year (Cross et al. 1991). Due to migration behaviour patterns of B.C. chinook stocks and to ocean troll fisheries along the North American coastline, part of chinook production from SEP hatcheries is caught by U.S. fishermen. The annual portion of B.C. hatchery chinook caught by Canadian fishermen during the 10-year period 1980-1989 ranged from 48.8% to 67.0%. Hatchery-produced coho salmon caught in fisheries from 1978-1989 ranged from 183.9 thousand to 649.5 thousand fish per year; over 90% of the coho salmon were caught by Canadian fishermen in all years (Cross et al. 1991).

The total numbers of juvenile salmonids cultured for ocean ranching in B.C. in 1989 was 724.4 million fish of which pink, chum, coho, sockeye, and chinook salmon represented 14.8, 31.8, 2.8, 41.9, and 8.9% respectively. In addition 0.29 million juvenile cutthroat trout (*Oncorhynchus clarki*) and 2.27 million steelhead trout (*O. mykiss*) were also released (DFO 1990). Not all of these latter two species, however, may have been released for ocean ranching.

### *Oregon and Washington*

The states of Oregon and Washington in the U.S. both have long standing ocean ranching programs dating back to the last century, and early hatcheries in both states have many similarities including mixed levels of initial success. Two important technological developments in the 1950s and 1960s, namely better knowledge of nutritional requirements resulting in better diets and new effective therapeutics for control of disease (Clever 1969), have led to improved performance and significant hatchery expansion in both states.

Expansion of hatchery-based ocean ranching in both Oregon and Washington was also related to another common factor, the need for mitigative replacement of salmonid production from extensive losses of natural habitat due to industrial development, urbanization, and especially to damming of the Columbia River and its tributaries. This boundary river between these states was originally one of the great salmon rivers of the world. Unfortunately, the river also had great hydroelectric potential that has been extensively developed. Mitigation of salmonid production in the Columbia River involves ranching programs by many groups including the states of Oregon, Washington, and

Idaho, the U.S. Fish and Wildlife Service, and several native American tribal groups under the umbrella of the Columbia River Inter-Tribal Fish Commission.

While a review of salmon ranching in Oregon (Lannan 1980) and Washington (Donaldson 1980) show many similarities; a unique distinction was that Oregon, in 1971, enacted legislation to allow for private hatcheries. Private-for-profit salmon ocean ranching was developed around the principle of substituting private capital for public funds to increase Oregon's overall salmon enhancement program. The concept allowed common property harvest of returning adults by recreational and commercial fisheries while fishing in traditional areas, and proprietary ownership, harvest and sale of fish returning to hatcheries or permitted release sites (Lannan 1980; Berg 1981). As reviewed by Gowan (1988), Oregon regulations required that smolts from private hatcheries be released into an estuary or the ocean, a concept designed to prevent conflicts with natural runs or with public hatcheries (Lannan 1980).

Berg (1981), citing unpublished data of Cummings (1979), identified 14 permitted private hatchery sites along the Oregon coast; some were authorized to release only one species and others up to three species. Apparently not all of these hatcheries actually made releases of juveniles due to shortages of suitable eggs (Lannan 1980; Berg 1981). In 1977 private hatcheries in Oregon released 13.7 million juvenile salmon, mostly coho (Lannan 1980), and by 1980 total releases apparently exceeded 30 million juveniles, primarily coho and chinook (Berg 1981). By 1988, however, releases had fallen to 14.7 million (CRITFC 1991). No private hatchery releases were made in 1990 and by the end of 1991 all but one private ocean ranching operation in Oregon has ceased to operate (J. Leppink, Ore. Dept. Fish. Wildl., pers. comm.).

In both Oregon and Washington ranching traditionally has focused more on coho and chinook salmon than on other species because these species have strong constituent support from both commercial and recreational fishery interests. In Washington, for example, roughly three-quarters of the 337.7 million juvenile salmonids released in 1987 were chinook or coho salmon. During that year releases made at 137 hatcheries or release locations totalled 139.4, 17.9, 88.4, and 90.2 million juveniles, respectively, for fall chinook, spring chinook, coho, and chum salmon (Abrahamson 1988). Remaining releases were mostly steelhead trout.

In Oregon during 1989 two-thirds of the 21.9 million juveniles released were chinook and coho salmon. Ocean-ranched salmonids released in Oregon in 1989 included, in millions, 6.6, 2.3, 5.7, 7.0 and 0.3, respectively, for fall chinook, spring chinook, coho, steelhead, and chum salmon (CRITFC 1991).

### *Alaska*

Like other areas, Alaska also has had two distinct periods of hatchery development. The first period, focused on sockeye salmon and development of early-day fish culture technology, was phased out by the late 1930s (Roppel 1982). The second phase began in the 1970s when Alaska's salmon harvest had reached record low levels (Figure 2). Also, successful ranching programs in other areas, notably Japan, Oregon and Washington, gave support to the idea for development of new hatcheries in Alaska.

In 1971 the Alaska Legislature created the Fisheries Rehabilitation, Enhancement, and Development (FRED) Division of the Alaska Department of Fish and Game (ADF&G) to operate public hatcheries and help rebuild depressed salmon runs. The program was expanded in 1974 authorizing operation of private non-profit (PNP) hatcheries, also to aid in rebuilding depressed salmon resources by contributing to traditional common property fisheries. Long-term low-cost state loans were provided for construction and initial operation of PNP hatcheries. Oversight and technical assistance for all ranching in Alaska is under ADF&G stewardship, supported by laboratories for fish health, genetics, lake enrichment, mark sampling and tag processing (Koenings 1993; McNair and Holland 1994).

PNP statutes also provided for regional aquaculture associations, comprised of representative fishery resource user groups within regions. These regional groups can: (1) build and operate hatcheries: (2) assist ADF&G in developing and maintaining regional salmon plans; (3) authorize tax assessments on common property commercially caught salmon to support ranching; and (4) provide for the sale of a portion of returning hatchery fish to help cover operational costs and repay state loans (McKean 1991). Initially both public hatcheries in Alaska, operated by FRED, and private hatcheries, operated mostly by Regional Associations, had similar though somewhat duplicative roles (Orth 1978). Now, however, operation of many FRED hatcheries, originally funded with state bonds, have been transferred to regional associations under long-term cooperative lease agreements with the state. Under these arrangements the associations are responsible for the cost of annual operations.

By 1992, eight regional aquaculture associations had formed in Alaska and five had either built hatcheries or were operating hatcheries initially built as public FRED facilities (McNair and Holland 1993). In 1993, the FRED and Commercial Fisheries Divisions of ADF&G were combined to form a new Commercial Fisheries Management and Development Division (McNair and Holland 1994).

Salmonid ranching in Alaska continues to evolve; it now represents an innovative blend of public and private sector participation. The concept of "nonprofit" hatcheries grew out of concern for preventing large corporations from gaining an entry into control of public resources. In the Alaska example, "profit" from ranching goes to common property harvesters in coastal fisheries, both commercial and recreational, for regional

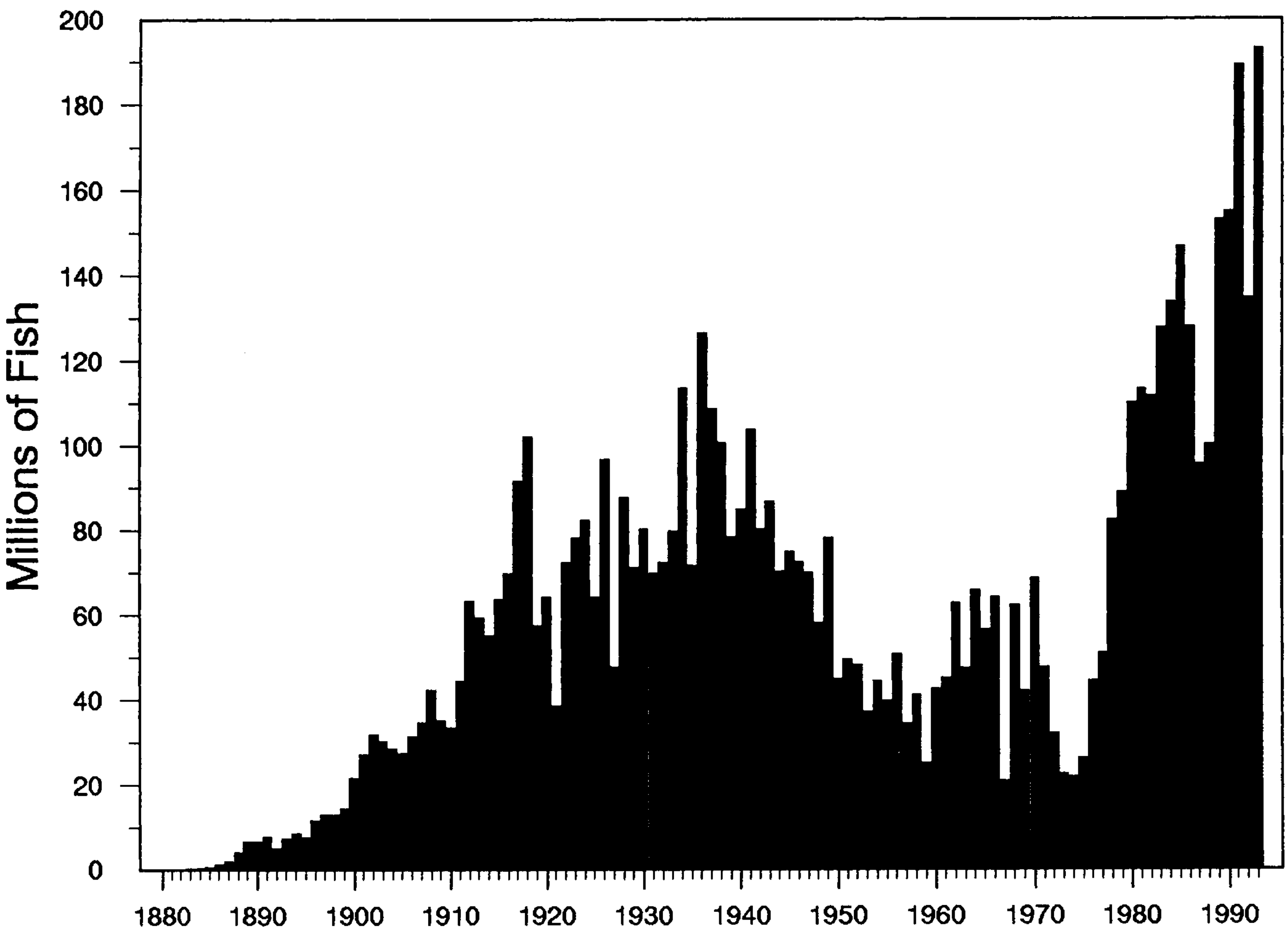

FIGURE 2. Alaska commercial salmon harvest from 1886 to 1993.

economic benefit. Likewise, the "private" component of Alaska's PNP program is also special since aquaculture associations that build or operate hatcheries, are quasi-public institutions with broad regional representation. Alaska's system, similar in many aspects to the fishermen cooperative system in Japan (Nasaka 1988), provides procedures for principal beneficiaries of ranching to help pay program cost. A significant difference between Japan and Alaska, however, is, that in addition to a large ranching program, the continued preeminent role of wild stock production in Alaska is not present in Japan.

In 1992 there were 38 active production salmon hatcheries operating in Alaska including one operated by a native American reservation on Annette Island in the southeast region (McNair and Holland 1993). In addition there were about 60 other scientific-educational ocean ranching permits in Alaska; most were associated with school district aquaculture programs; two involved federal research hatcheries.

Releases of ocean-ranched salmonids in Alaska during the 5-year period 1988-1992 has exceeded 1 billion juveniles annually with over 90% consisting of pink and chum salmon (Table 2). A significant portion of all ranched salmon in Alaska is located within the Prince William Sound (PWS) region where five major hatcheries operate (Figure 3). One hatchery in PWS is operated by the Valdez Fishery Development Foundation and four are operated by the Prince William Sound Aquaculture Corporation (PWSAC), including the largest hatchery in the state at Ester Lake (Figure 4).

In the 5-year period 1988-1992 the number of ranched salmon returning to Alaska programs ranged from 19.5 to 48.5 million adults (Table 3). Only a portion of ranched salmon in Alaska, roughly 65%, are caught in commercial fisheries; others are harvested in recreational fisheries, some are required for hatchery brood, and still others harvested by PNP groups to help cover cost of hatchery operations.

Although contemporary ranching began in Alaska during a period of depressed natural production, wild salmon runs have since rebounded to historic levels. During the 14-year period, 1980 to 1993, total commercial salmon harvest in Alaska exceeded 100 million adults in all but one year (Figure 2); commercial catch in 1993 was a record high 193 million fish.

## PLANNING ASSUMPTIONS FOR OCEAN RANCHING

Ocean ranching can be placed in three broad planning processes focused on biological, economical and societal issues. Through planning these issues determine the feasibility of programs and individual projects. Time sequences between planning and production, often several years in length, concomitant with changes that may occur in initial assumptions, can change the overall feasibility of projects (Shepherd 1991).

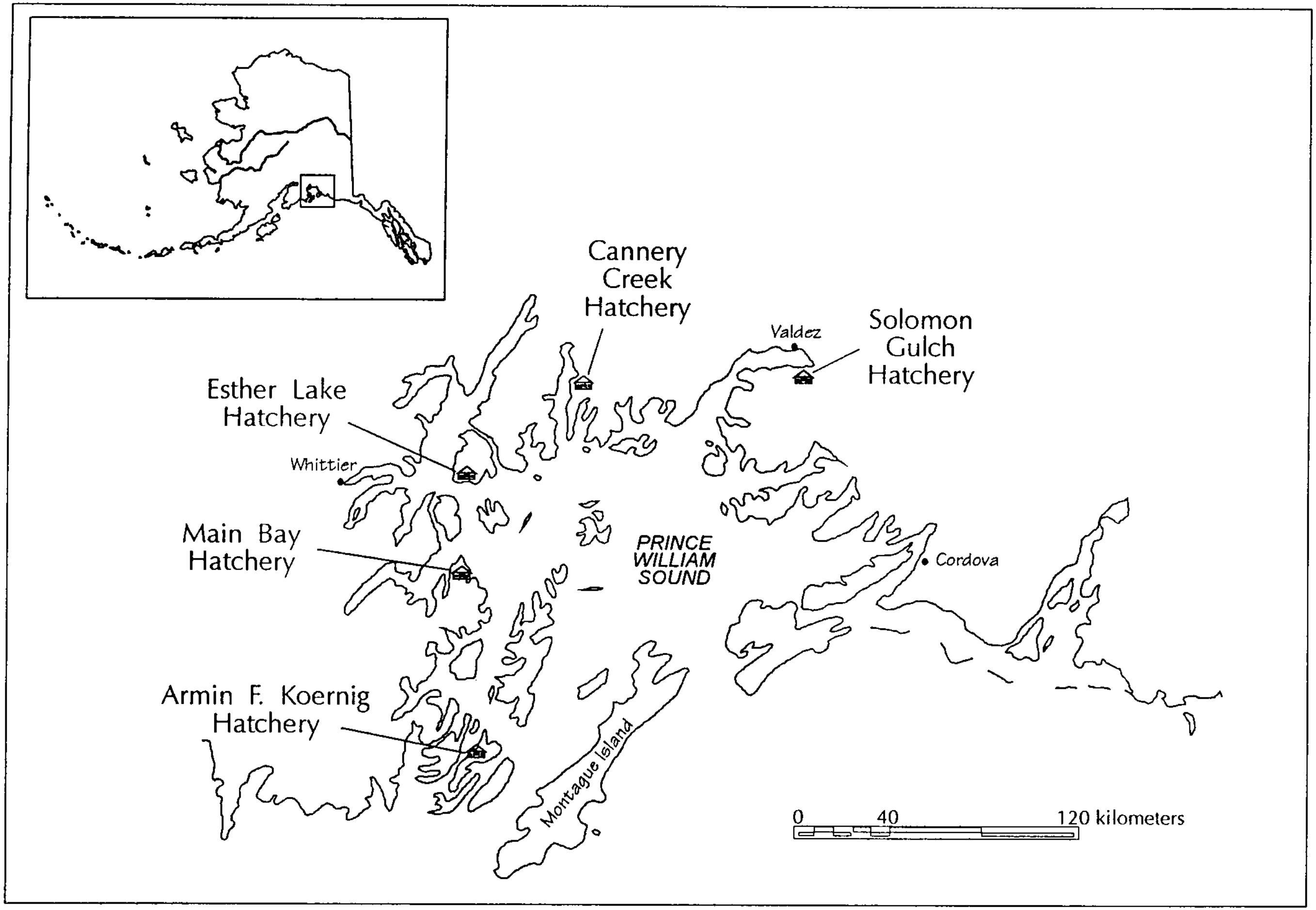

FIGURE 3. Prince William Sound region of Alaska and location of five production salmon hatcheries operated by aquaculture associations.

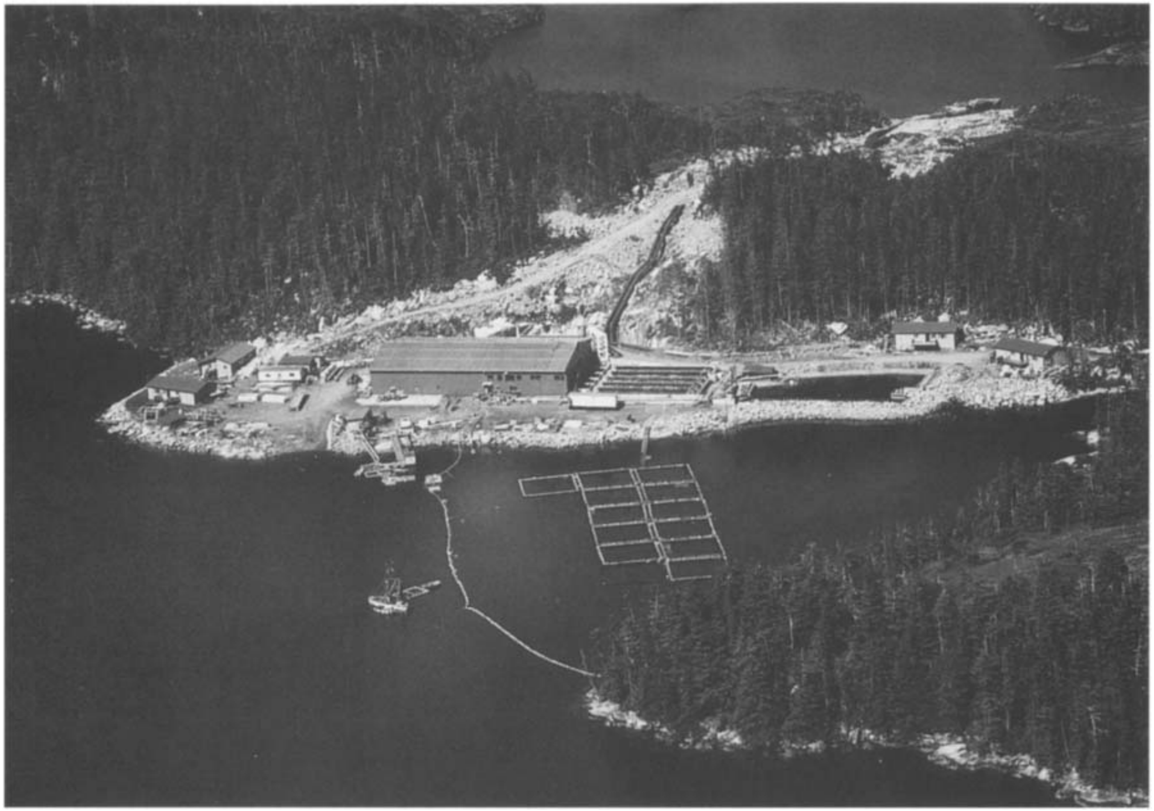

FIGURE 4. Wally Noerenberg Hatchery (Ester Lake) at Ester Island, the largest production facility in Prince William Sound, with a capacity to incubate up to 300 million salmon eggs. Water for the hatchery originates from Ester Lake in the background which is not accessible to anadromous fishes due to barrier falls. Photo by Prince William Sound Aquaculture Corporation (PWSAC).

TABLE 2

Releases of juvenile salmon (× 1000) from Alaska hatcheries, 1988-1992 [1].

| Year | Pink | Chum | Coho | Chinook | Sockeye | Total |
|---|---|---|---|---|---|---|
| 1988 | 839,400 | 292,581 | 19,161 | 6,325 | 69,142 | 1,222,609 |
| 1989 | 672,490 | 308,882 | 16,225 | 8,021 | 77,281 | 1,082,899 |
| 1990 | 808,308 | 242,581 | 17,958 | 10,137 | 73,388 | 1,152,371 |
| 1991 | 861,978 | 373,892 | 16,119 | 7,284 | 68,984 | 1,328,257 |
| 1992 | 801,770 | 434,198 | 14,625 | 9,818 | 75,125 | 1,335,536 |

[1] Data from Alaska Department of Fish and Game, FRED Division annual reports.

Recent world-wide rapid growth in production of farmed salmon (Folsom et al. 1993), for example, has saturated many traditional markets, creating surpluses and depressing wholesale prices, along with disruption of established marketing patterns. In Alaska salmon harvests accounted for 41% of world salmon markets in 1980. By the end of 1990, wild and ranched Alaska salmon accounted for only about 30% of world supply even though a 50% increase in Alaska salmon production occurred during the same period (ASMI 1991). Societal factors generally integrate biological and economic issues within a regional context to determine if projects are implemented. In some cases a project may be biologically or economically marginal but still provide other unique societal values like local employment or a focal point for community development (DFO 1989*a*). Ranching efforts to restore depressed, threatened or endangered natural runs may produce non-economic decisions where cost-benefit factors become secondary to other biological and societal issues (Hard et al. 1993).

### *Biological Planning*

Biological criteria should consider all elements of modern salmonid hatchery systems, in addition to how well ranched fish may fit into the ecology of affected areas. Ranching should not create new environmental problems. Facility siting and the release of the best quality juveniles are two crucial biological planning elements (Shepherd 1984). Careful attention to facility layout including details of rearing and incubation systems are essential (PWSAC 1975). Other important bioengineering issues include water quality and sources, rainfall and weather patterns, species and stocks chosen for culture and close scientific supervision of any pilot experimentation at proposed sites (Shepherd 1991). Proximity of hatcheries to wild stocks and details of harvest strategies for both ranched and wild fish are crucial issues. Harvesting ranched fish should not impose unacceptable exploitation levels on adjacent or intermixed wild stocks.

TABLE 3

Estimates of adult salmon returns (× 1000) from ocean ranching programs in Alaska, 1988-1992.

| Year | Pink | Chum | Coho | Chinook | Sockeye | Total | Source |
|---|---|---|---|---|---|---|---|
| 1988 | 14,581 | 2,637 | 291 | 72 | 1,891 | 19,472 | Holland (1989) |
| 1989 | 31,771 | 1,387 | 529 | 75 | 2,181 | 35,943 | Holland (1990) |
| 1990 | 41,208 | 2,010 | 888 | 111 | 4,329 | 48,546 | McKean (1991) |
| 1991 | 38,132 | 2,260 | 1,258 | 122 | 6,374 | 48,146 | Holland and McKean (1992) |
| 1992 | 14,879 | 3,193 | 1,280 | 122 | 3,899 | 23,373 | McNair and Holland (1993) |

Along with planning details for major facilities, careful attention should also be given to remote sites where juveniles are released away from a principal hatchery. Remote off-station releases are an integral part of some ranching programs including those in Alaska, Sweden, and Washington. Off-station releases may be done to develop less congested terminal fisheries, to create other fisheries for special purposes (Eriksson 1991; Eriksson and Eriksson 1991), or for biological reasons such as avoiding predator concentrations near hatcheries (Hvidsten and Mokkelgjerd 1987; Gunneröd et al. 1988). Another form of off-station releases, often called outplanting or supplementation, is intended to enhance natural stocks (Winton and Hilborn 1994). In Alaska off-station releases generally follow a vernal period of short-term rearing in marine netpens for imprinting, acclimation, and final growth before juveniles are released (Josephson and Kelley 1993). These releases are often made near non-anadromous freshwater streams to facilitate imprinting.

Due to potential interactions between ranched and wild salmonids, including possible straying of unharvested ranched fish into wild streams, off-station releases should be treated and evaluated as separate entities, independent of any association with a parent facility. This is especially applicable to so-called central incubation hatcheries where several stocks or species may be incubated and reared to early fry stage before release at other locations. While these practices may provide favourable economic potential, they also carry additional biological risk.

Post-release biology involving migration behaviour, feeding habits, survival, and homing fidelity determine, in large measure, performance and ultimate success of ranched salmonids. One critical biological assumption is that performance of ranched juveniles can, at the release stage, behaviourly and physiologically approximate that of wild fish.

Ranching also generally assumes that the cultured fish can adjust rapidly to the wild environment and develop normally within it (*see* Chapter 5), that homing of ranched stocks will be reasonably accurate, and that the marine environment can support the additional ranched fish in addition to existing wild stocks (Thorpe 1986, 1988).

### *Economical Planning*

Simplistic economic models for ocean ranching involve long-term amortization of capital cost, annual operating expenses, scaled for different production levels, and, importantly, realistic assumptions for survival rates of juveniles released and recovered value of adults produced. Common property fisheries, shared among different resource users, have different economic projections than private-for-profit ranching efforts. Migratory pathways of returning adults, with even moderate fishery interceptions prior to arrival in natal regions, become important components of an overall economic outlook.

Along with cost of producing young, estimates of marine survival (numbers of returning adults) and recovered value determine economic feasibility of most projects (PWSAC 1975; Hartman 1986). Projected adult returns become guidelines for harvest strategies including percentages of ranched fish: (1) caught in common property fisheries; (2) available for cost recovery harvest by hatchery operators; and (3) escapement for future brood. Initial estimates of returns, often based on average survival values for different species in a given region, are refined later to reflect actual performances.

Marine survivals may fluctuate significantly without clear indications of causal factors over a few years, with important favourable or unfavourable economic consequences to ranching efforts. Walters (1988) reviewed present limitations and proposeed new experimental approaches to test several hypotheses to explain the theory that hatchery productivity often declines after initial higher survivals. Most of the experiments suggested by Walters (1988) remain to be done.

While there are examples of declining hatchery survival following higher initial performance (Hilborn 1992), the reverse is also true and survival can also increase after periods of low hatchery productivity (Figure 5). A weakness in some assessments comparing natural production with ranching performance is a fallacious assumption that wild stock production remains constant (Hilborn and Winton 1993), and that only hatchery stocks fluctuate. Unfortunately, however, such assumptions are often necessary because precise survival data and other detailed parameters collected at hatcheries are not available for most wild stocks.

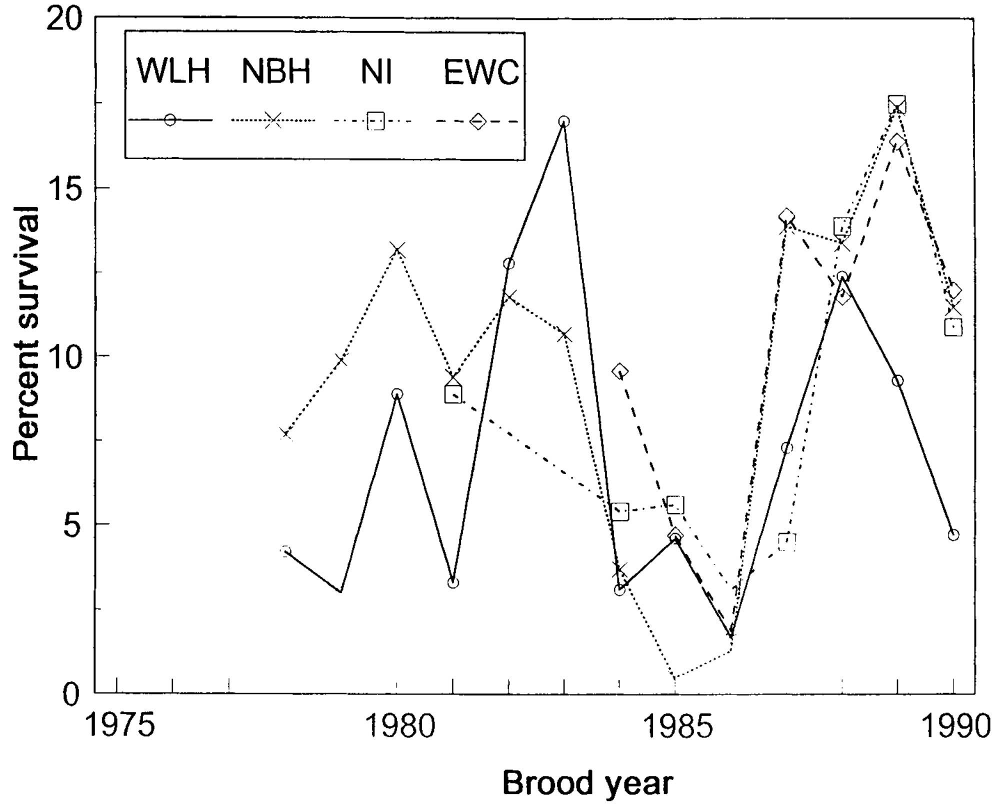

FIGURE 5. Marine survivals including catch and escapement for coho salmon from four ocean ranching facilities operated by Southern Southeast Regional Aquaculture Association (SSRAA) in Ketchikan, Alaska. The same stock, originally from Indian Creek-Chickamin River, is used at each facility. WHL, Whitman Lake Hatchery; NBH, Neets Bay Hatchery; NI, Naket Inlet; EWC, Earl West Cove. Naket Inlet and Earl West Cove are remote release facilities.

## ECONOMIC VIABILITY OF RANCHED SALMONIDS

Economics of salmonid ocean ranching is a function of numerous complex issues that vary widely in different countries. Often viewed only in the context of commercial fish sales for market, recreational fisheries and associated activities can play a major role in the economic viability of ranching. For example, in Iceland, based on the lack of coastal commercial fisheries and the proprietary recreational use of ranched fish in Islandic rivers, Isaksson (1989) stated, "Ocean ranching for the export of salmon is only marginally economical in Iceland but ranching to enhance sport fisheries is highly profitable". In contrast, sport fishing in Japan is only a marginal factor, yet the Japanese operate perhaps the most economically viable salmon-ranching program in the world (Sano 1995). Many programs in North America, based on a variable mixture of sport fishery and commercial fisheries values and interest likely would fall somewhere between these extremes (Figure 6).

Ranching programs focused on species with a relatively short freshwater life stages, such as pink or chum salmon, may have the best chance for profitable operations (Isaksson 1989; Sano 1995). Average cost of producing different species of juvenile salmon in Southeast Alaska over a 5-year period (1988-1992) show a range of \$0.016 - \$0.024 US per chum salmon juvenile and \$ 0.323 - \$0.418 US per yearling chinook and coho salmon juvenile produced (Table 4). Age-0 chinook salmon were comparable in cost to chum salmon. The same species cultured at different hatcheries operated by the same aquaculture association had different production costs based on features specific to each hatchery. Cost of adult salmon produced, based on average marine survivals and 1992 cost of raising juveniles in southeast Alaska ranged from \$0.54 US per fall chum salmon to \$11.26 US per chinook salmon (Table 5).

In Japan the cost of producing chum salmon during the period 1962-1972 was estimated at ¥ 1.94 (yen) per fry, and ¥ 85.4 per adult (Kobayashi 1980). Roughly a decade later (1983 brood), Sano (1995) estimated the cost of producing chum salmon at about ¥ 4 per fry released and ¥ 194.8 per adult recovered. An average price of ¥ 635 per kg paid for adult chum salmon, weighing an average of 3.5 kg in 1987, provided a "profit" to the Japanese ocean-ranching program of ¥ 2222.5 per adult caught (Sano 1995). It was not clear if these calculations included amortization of facility cost. By 1991, however, Kaeriyama and Urawa (1993) document a dramatic decline in the price paid for chum salmon to fishermen on Hokkaido with more than a 20% drop in price from 1990 to 1991.

Economic viability of salmonid ranching depends ultimately on value of fish produced and value is essentially a product of world market forces. In Alaska, for example, the ex-vessel value of commercial salmon harvest has fluctuated greatly during the period 1988-1992 in spite of record catches (Figure 7). During this same period ranched fish comprised from 16 to 30% of the total catch.

Extreme changes in ex-vessel prices paid to commercial fishermen in Southeast and PWS Alaska by species and gear type can be seen in comparing 1988 and 1992 values (Table 6). Average prices paid for purse seine caught pink salmon in PWS, for example, were \$1.87 US per kg in 1988 versus \$0.44 US per kg in 1992, a 76% decline in value over the 5-year period. Such sharp declines in value cast a cloud of economic uncertainty on the large ocean-ranching program for pink salmon in PWS. Armin Koerning, one of the pioneers in development of the PWSAC program, indicated, for their pink salmon ranching program to be successful, prices needed to average \$0.55 US per kg or more (A. Koerning, pers. comm.).

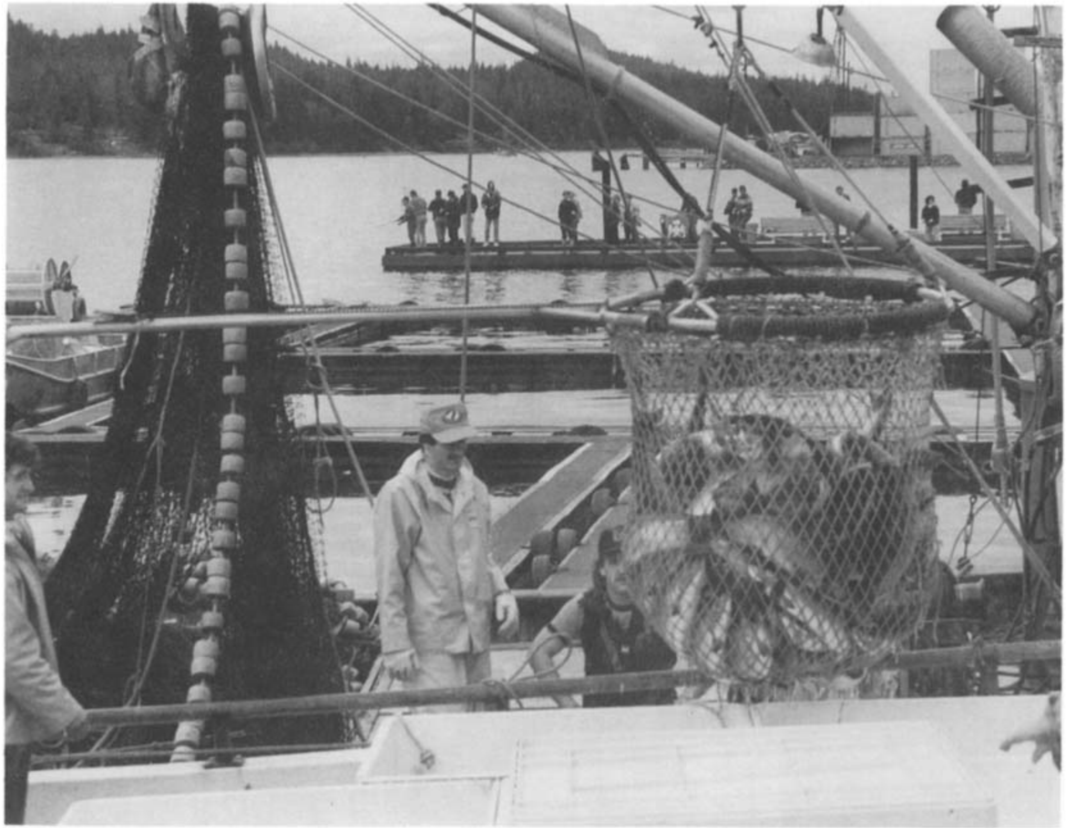

FIGURE 6. Cost-recovery coho salmon being brailed from a purse seine operation (foreground) at the private, non-profit Gastineau Hatchery operated by Douglas Island Pink and Chum, Inc. in Juneau, Alaska. A common property, no-fee, sport fishery for salmon returning to the hatchery from a floating dock is in the background. Photo by author.

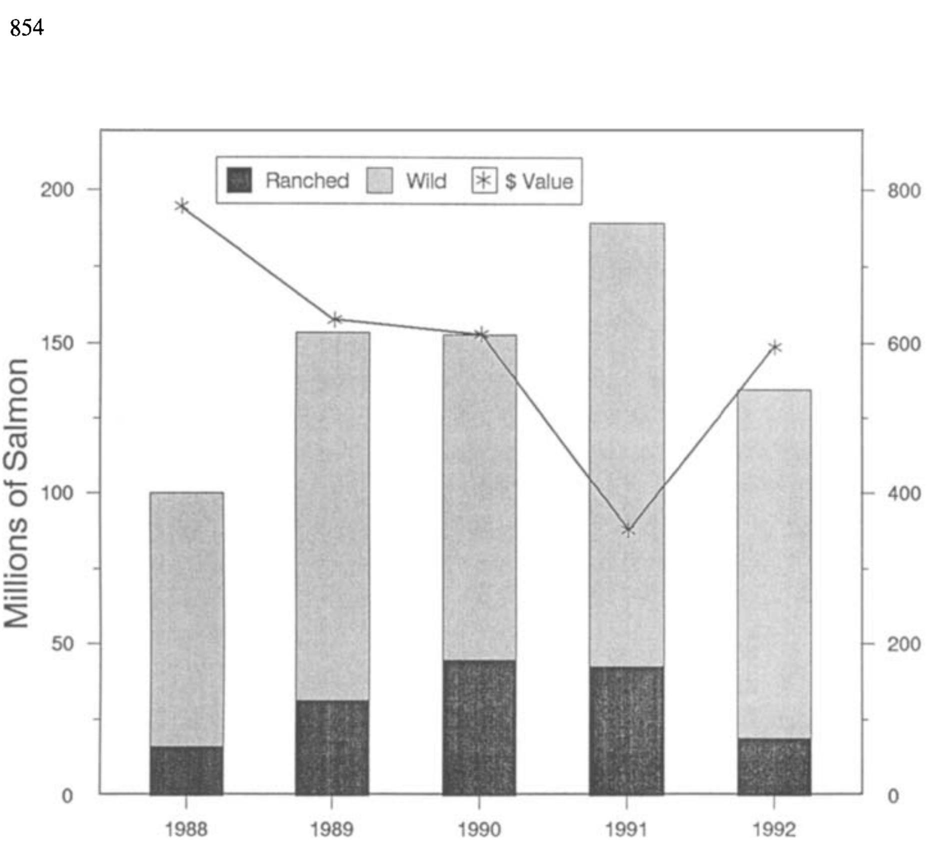

FIGURE 7. Total numbers of commercially caught wild and ocean-ranched Alaska salmon, 1988-1992, and annual ex-vessel value of the catch. Ranched salmon include both common property and cost recovery harvest. Data from Alaska Department of Fish and Game and Commercial Fisheries Entry Commission.

Although the primary purpose of PNP programs in Alaska is to produce adult salmon for common-property harvest, individual hatcheries harvest and sell up to 30% of their returning fish. Designated harvest zones near hatcheries or at remote release sites provide either for common-property or for cost-recovery fisheries. Allocating common-property fisheries of ranched salmon among different types of fishers (seine, gill net, troll, sport), may be based on restricting the harvest to only one gear group at specific hatcheries (PWSAC 1990*a*), or rotating access among different gear groups throughout the fishing season (SSRAA 1994).

A benefit-cost analysis of the Alaska ocean ranching by Boyce et al. (1992) indicated that, over a three decade-long period 1992-2021, in many regions and with most species, a reduction or elimination of the state's enhancement program would provide greater economic benefit than continuing or expanding present levels. The analyses only

TABLE 4

Annual cost[1] (US$) per smolt at two hatcheries operated by a regional aquaculture association in Southeast Alaska, 1988 to 1992.

| Hatchery and species | Age at release [2] | 1988 | 1989 | 1990 | 1991 | 1992 | Weighted 5-year average |
|---|---|---|---|---|---|---|---|
| **Neets Bay** | | | | | | | |
| Coho | 1+ | 0.369 | 0.327 | 0.259 | 0.417 | 0.357 | 0.323 |
| Chinook | 1+ | 0.485 | 0.435 | 0.295 | 0.472 | 0.403 | 0.414 |
| Chinook | 0+ | ------- | ------- | ------- | 0.141 | ------- | 0.120 |
| S. Chum[3] | 0+ | 0.027 | 0.023 | 0.019 | 0.027 | 0.023 | 0.024 |
| F. Chum[4] | 0+ | 0.020 | 0.019 | 0.014 | 0.016 | 0.012 | 0.016 |
| **Whitman Lake** | | | | | | | |
| Coho | 1+ | 0.395 | 0.356 | 0.353 | 0.291 | 0.414 | 0.346 |
| Chinook | 1+ | 0.454 | 0.420 | 0.289 | 0.349 | 0.418 | 0.370 |
| Chinook [5] | 1+ | 0.490 | 0.450 | 0.346 | 0.385 | 0.450 | 0.418 |
| Chinook [5] | 0+ | ------- | ------- | 0.248 | ------- | ------- | 0.188 |

Source: SSRAA Spawning News 14(3)

[1] Cost includes debt service, which on average across all species and facilities is about 30% of total cost.

[2] Age 1+ or yearling smolts are typically released in spring or early summer following 12 to 16 months of post swim-up fry stage feeding in the hatchery. Age 0+ smolts are typically released following 30 to 90 days of post swim-up fry stage feeding in the hatchery or in marine netpens.

[3] S. Chum = summer chum salmon

[4] F. Chum = fall chum salmon

[5] Remote release site at Carroll Inlet.

considered the economics of commercial fisheries. Other resource users such as sport and subsistence fishermen were not included; neither were indirect benefits like employment and income in fish processing and other support services. This analyses also suggested the most cost-effective programs, rather than being subsidized by the state, would result from those funded entirely by user groups within regions. While this process is already underway by shifting more responsibility for programs to regional aquaculture groups, other procedures and conclusions in the Boyce et al. (1992) analyses have come under sharp criticism (Knapp 1993; Wilen 1993). In contrast to the Boyce et al. (1992) study, Allee (1988) estimated that expenditures on Alaska's ranching program produced

TABLE 5

Average cost of smolt production in 1992 at two hatcheries operated by a regional aquaculture association in southeast Alaska; estimated number of returning adults, and cost of producing each adult salmon.

| | | Cost [1] | | Expected adults | |
|---|---|---|---|---|---|
| **Hatchery and species** | **Smolts produced (N)** | **Total (US$)** | **Per Smolt (US$)** | **Total [2] (N)** | **Cost/Adult (US$)** |
| **Neets Bay** | | | | | |
| Coho | 2,303,000 | 822,540 | 0.357 | 200,361 | 4.11 |
| Chinook | 728,500 | 293,193 | 0.403 | 29,140 | 10.06 |
| Chum (summer)[3] | 23,292,000 | 523,873 | 0.023 | 582,050 | 0.90 |
| Chum (fall) | 25,202,000 | 310,495 | 0.012 | 579,846 | 0.54 |
| **Whitman Lake** | | | | | |
| Coho | 303,300 | 126,047 | 0.414 | 21,605 | 5.83 |
| Chinook | 106,200 | 44,417 | 0.418 | 4,248 | 10.46 |
| Chinook [4] | 1,217,800 | 548,513 | 0.450 | 48,712 | 11.26 |

Source: SSRAA Spawning News 14(3)

[1] Cost includes debt service which, on average across all species, equals about 30% of total cost.

[2] Based on average survival rates for the hatchery and species.

[3] Summer and fall chum salmon runs are based on different races that enter fisheries and spawn during different time periods.

[4] Remote release site at Carroll Inlet.

two to four times the level of positive impacts that result from similar expenditures on other state operating or capital budgets.

Hilborn and Winton (1993), evaluating SEP in Canada, concluded the program had fallen short of its stated goals. Effectiveness of the three main SEP technologies: hatcheries, spawning channels and lake enrichment, according to these authors, cannot be determined after 15 years of operation due, in part, to variations in survival rates. They suggested that perhaps 50 years might be more appropriate for learning when and where specific technologies will work best. They also recommended outside professional oversight to evaluate enhancement programs and posed unanswered questions on potential interactions between SEP and natural-spawned fish.

TABLE 6

Comparative ex-vessel prices paid to Alaska commercial salmon fishermen in 1988 and 1992 in the Southeast and Prince William Sound regions by species and harvest gear. Values listed are in U.S. dollars per kilogram and, in parentheses, U.S. dollars per fish.

| | | Region | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Southeast | | | | Prince William Sound | | | |
| Species | Harvest gear | 1988 | | 1992 | | 1988 | | 1992 | |
| Chinook | Troll | 8.39 | (70.09) | 5.36 | (38.15) | [1] | | ------ | |
| | Purse Seine | 5.53 | (52.19) | 1.94 | (14.02) | 5.44 | (64.91) | 2.51 | (28.25) |
| | Gillnet | 4.14 | (27.44) | 2.68 | (18.34) | 7.24 | (86.31) | 6.10 | (68.54) |
| Coho | Troll | 6.81 | (23.79) | 3.33 | (10.58) | ------ | --------- | | |
| | Purse Seine | 4.34 | (14.99) | 1.65 | ( 5.09) | 3.99 | (17.47) | 1.81 | ( 7.11) |
| | Gillnet | 5.25 | (21.15) | 2.07 | ( 8.06) | 4.42 | (19.36) | 2.07 | ( 8.61) |
| Sockeye | Troll | 7.61 | (18.66) | 3.75 | ( 8.48) | ------ | --------- | | |
| | Purse Seine | 6.66 | (16.31) | 3.66 | ( 9.82) | 5.77 | (16.09) | 3.67 | ( 9.96) |
| | Gillnet | 6.81 | (20.40) | 3.95 | (11.28) | 6.76 | (18.86) | 4.76 | (12.92) |
| Chum | Troll | 4.25 | (13.52) | 1.23 | ( 3.60) | ------ | --------- | | |
| | Purse Seine | 2.29 | (12.06) | 1.12 | ( 3.99) | 2.34 | ( 9.09) | 0.99 | ( 3.51) |
| | Gillnet | 2.55 | (11.46) | 1.03 | ( 4.18) | 2.38 | ( 9.24) | 1.53 | ( 5.41) |
| Pink | Troll | 2.16 | ( 2.85) | 0.70 | ( 0.89) | ------ | --------- | | |
| | Purse Seine | 1.85 | ( 2.68) | 0.51 | ( 0.76) | 1.87 | ( 2.95) | 0.44 | ( 0.66) |
| | Gillnet | 1.87 | ( 3.30) | 0.46 | ( 0.80) | 1.84 | ( 2.91) | 0.45 | ( 0.69) |

Source: Catch and sales statistics, Alaska Department of Fish and Game and Commercial Fisheries Entry Commission.

[1] There is no commercial troll fishery in the Prince William Sound region.

## INTERACTIONS OF RANCHED AND WILD SALMONIDS

Wild-hatchery salmonid stock interactions are paramount issues among scientists studying these fishes on both east and west Atlantic and Pacific Ocean coastlines. In recent years numerous conferences, workshops and international symposia have addressed this matter. Concerns center largely around two themes; over-harvesting of weak wild stocks in mixed stock fisheries where hatchery fish can withstand higher exploitation rates, and biological interactions with potentially adverse impacts on wild stocks. Potential adverse biological interactions include loss of genetic diversity (inbreeding depression), loss of fitness (outbreeding depression), transmission of diseases or parasites, predator concentrations, competition for food or habitat, and failure to maintain other unique, stock-specific features in wild fish that interbreed with cultured fish (Hanson 1988; Walters 1988; Waples et al. 1990; Hindar et al. 1991; Waples 1991; Gharrett and Smoker 1993).

The difficult task of quantifying biological interactions between cultured and wild salmonids is presently the focus of ongoing research in many countries. Hinder et al. (1991) observed "where genetics effects on performance traits have been documented, they always appear to be negative in comparison with unaffected native populations". However, in reviewing supplementation hatchery practices for chinook salmon in British Columbia, Winton and Hilborn (1994) noted "....a paucity of information on wild stocks and the extent to which...enhancement activities have an effect on their numbers". Accurate data on natural populations is essential to measure potential effects of genetic and biological interactions with hatchery-reared fish.

Minimizing adverse effects of mixed hatchery-wild stock fisheries may include careful attention to hatchery location, including off-station releases, creative fisheries management to separate stocks as much as possible, and designating regions, special areas, or water bodies for management as exclusive hatchery or wild stock zones.

### *Iceland*

In Iceland, Gudjonsson (1991) documented proportions of wild salmon along with strays from farmed and ranched fish in four rivers during the period 1986-1989. He used distinctive scale patterns to separate the different groups of fish. Wild fish tended to enter the rivers earlier than the reared strays; yearly percentages of cage-reared escapes ranged from none to 63.0%, and of ocean-ranched strays ranged from 2.0% to 8.2% of the fish sampled. The highest occurrence of reared fish reported was in River Botnsa in 1988 when 63.0% and 6.0% of fish sampled between July and September were from cage-reared and ranched strays, respectively.

Recent new Icelandic regulations to reduce the genetic threat of reared strays on wild stocks include: (1) the use of native stock for enhancement in salmon rivers; (2) cage-rearing sites must be a minimal distance from the estuary of salmon rivers; and (3) coded-wire tagging is required of some portion of all smolts used in ranching and cage-rearing programs. It was also suggested that ocean ranching should only use stocks from rivers close to the ranching site and that operations build up slowly (Gudjonsson 1991).

### *Norway*

Atlantic salmon smolts and parr were released in northern and mid-Norway streams from a variety of stocks including transplants from other countries until the early 1980s (Heggeberget and Johnsen 1982; Johnsen and Jensen 1991). Baltic salmon transplants from Sweden around 1975 are now believed to be the source of infestations of an external parasite, the monogenean *Gyrodactylus salaris*, that by the end of 1989, was present in 34 Norwegian rivers and in some had seriously depleted salmon populations (Johnsen and Jensen 1991). A new ranching policy in Norway now calls for only using local stocks for releases of cultured smolts, part of a broader set of international recommendations for minimizing threats to wild salmon stocks from cultured fish (NASCO 1991).

Much ranching research in Norway is now focused on behavioural aspects of hatchery-reared smolts in comparison with wild smolts in an attempt to minimize possible negative effects on natural populations (Hansen et al. 1984; Hansen and Jonsson 1985, 1986, 1989, 1991; Heggberget et al. 1991; Jonsson et al. 1991). Some studies were specifically designed to shed light on impacts of escapees from sea farming on wild salmon (Hansen et al. 1987), while others have examined aspects of marine survival relative to predation (Hvidsten and Mokkelgjerd 1987) and release time or location (Gunneröd et al. 1988; Hansen et al. 1989; Heggberget et al. 1991).

### *Sweden*

In a protocol for preserving depleted wild stocks in the Baltic Sea, Eriksson and Eriksson (1993) combine creative management with off-station releases to more closely focus fisheries on predominantly hatchery fish. Delayed releases into coastal Baltic waters modifies hatchery smolt migration patterns so they are largely separated from wild stocks. A management scheme is proposed to restrict the offshore fishery to inside 24 nautical miles within the Main Basin where the delayed releases are made (Eriksson 1991; Eriksson and Eriksson 1991, 1993; Torleif Eriksson, pers. comm. 1991). Elsewhere, delayed releases of Pacific salmon also have resulted in modified migration patterns and residualization in local waters (Moring 1976; Novtony 1980).

Research on the effects of age and timing of delayed-released smolts in coastal Baltic waters suggests good survivals and contributions to fisheries without significant increases in straying into rivers (Eriksson 1989*a*, 1989*b*; Eriksson 1991; Eriksson and Eriksson 1993). Theoretically this management approach could allow development of a coastal fishery while protecting wild stocks in the Main Basin and permitting selected harvest of river-released stocks in more terminal areas. The idea is complicated, however, by an urgent need for international cooperation. Failure to effectively regulate a seven-nation fishery in the Main Basin has threatened the continued existence of the few remaining wild stocks in the Baltic Sea (Eriksson and Eriksson 1993).

### *Japan*

The successful ranching program in Japan results from an almost total commitment to hatchery production, especially with chum and pink salmon. Wide transplantation of hatchery chum salmon between rivers on Hokkaido and Honshu, however, has raised concerns over maintaining genetic discreetness of different populations (Okazaki 1982). Kaeriyama (1989*a*) identified "genetic preservation and breeding for preservation of original populations...." as an important future goal of salmon ocean ranching in Japan. In studies with reciprocal transplants of masu salmon between Hokkaido rivers, Mayama (1989) and Mayama et al. (1989) have shown the importance of using local stocks for ranching to prevent disruption of important adaptive gene complexes in specific populations.

### *Pacific Northwest*

Waples (1991) reviewed causes of genetic interactions between wild and hatchery salmonids and presents strategies for minimizing genetic risk. Recent detailed attention to these matters in the Pacific Northwest of North America is due to the listing of several depressed wild salmon stocks as threatened or endangered under the Endangered Species Act of the United States.

Cramer (1996) documented the demise of depressed wild coho salmon stocks in the lower Columbia River due, in part, to high ocean and in-river fisheries harvest of abundant hatchery production from the same region. In this instance, brood stock selection, stock transfers between hatcheries, and high harvest rates on hatchery fish modified migration and run timing patterns so that hatchery runs now are 4-8 weeks earlier than original natal populations, and remnant wild runs in one tributary are 2 months later.

### *Alaska*

During the 1988-1992 period commercial salmon harvest in Alaska's PWS varied between 47 and 60% and averaged 56% of annual ranched salmon production in the state. In the PWS region proper, ranched fish have averaged about 75% of commercial harvest since 1988. The peak proportion (88%) of ranched pink salmon in the PWS commercial harvest in 1989 was anomalous due to modified harvest management required after the *Exxon Valdez* oil spill.

Eggers et al. (1996) reviewing trends of wild-hatchery pink salmon interactions in PWS through 1990 raised concerns over recent declines in wild stocks and the ability to successfully conduct fisheries on such large hatchery components concomitant with achieving required wild stock escapements. In an update on this issue, Kron (1995) noted that new data suggested earlier wild stock escapements may have been higher than previously thought, and that current wild stock escapements are still consistent with long term, pre-hatchery levels. He further noted that 1960-1976 pre-hatchery commercial pink salmon harvest in PWS averaged 3.3 million fish annually, while the annual 1977-1992 harvest, including both wild and hatchery fish, averaged 19.7 million pink salmon.

The extent of hatchery development in different parts of Alaska is contrasted by Bachen (1991) who found in 1990 that although 77.4% of commercial salmon harvest in PWS was from ranching programs, ranching in other regions, specifically Southeast, Cook Inlet, and Kodiak, only comprised 3.8%, 9.2% and 11.4%, respectively, of commercial salmon caught in those regions. Concerning hatchery-wild stock interactions, he also suggested "...the challenge is to select a biologically sound strategy with a high probability of success" and.... "projects that will yield targeted harvest rates without disrupting wild stock fishery management". An example of these principles is seen at Hidden Falls Hatchery in the Southeast region where ranched chum salmon have helped raise commercial catch of that species to the highest levels in 70 years, apparently with minimal impacts on wild stocks (Bachen and Linley 1995).

## DISCUSSION

Ocean ranching of salmonids is widely practiced on both coastlines of the North Atlantic and North Pacific Oceans and adjacent seas. Some ranching also occurs in mid-latitudes of the South Pacific. Ranching programs that support major commercial and recreational fisheries in many countries released roughly 4.5 billion juvenile salmonids annually in the 1980s (McNeil 1988). World-wide releases in the 1990s are now thought to exceed 5.5 billion juveniles annually. Ranching presently is conducted primarily by governmental or quasi-public institutional entities. The most significant private salmonid ranching apparently is in Iceland where economic incentives are directed at recreational fisheries. Both Japan and Alaska have major systems where aquaculture cooperatives or associations of commercial fishers, the principal beneficiaries, pay at least part of program cost.

Development and decline of private salmon hatcheries in Oregon was associated with considerable public controversy. Many ecological and socio-economic objections were raised relative to potential negative biological impacts of private ranched salmon either on wild stocks or public hatchery stocks. Fear of potential market domination from monopolistic corporate influences on traditional public fishery resources was also an issue in Oregon (Lannon 1980; Berg 1981). In Alaska similar fears led to the private non-profit principle. Failure of Oregon's private hatcheries, however, appears mostly related to bio-economic issues, specifically to lower than expected survival rates.

In Norway, Sweden, Japan, Washington, and Oregon much of the original habitat that made historic levels of salmonid production possible is seriously compromised. Fisheries in these areas now are heavily dependent on ocean ranching and, in most cases, likely will continue in the future. In many areas fisheries that initially focused on natural runs of salmon now contain complex mixtures of both wild and hatchery stocks originating from widely separate regions, often from different countries. Effective management to protect depressed wild fish and harvest available ranched fish is a complex undertaking that until recently has not received attention.

The lower Columbia River coho salmon paradox, relative to hatchery-wild stock interaction, poses an important question relative to the future of salmonid ranching. Sustained perturbations of habitat and over-fishing in the first half of the century reduced wild coho salmon in the lower river to near extinction. Then hatchery-based ranching programs restored abundance of the species to historic levels. Restored runs, however, were not the same as original endemic ones, and continued fisheries along with biological changes in populations have now rendered wild coho salmon in the area even closer to extinction (Johnson et al. 1991; Cramer 1996).

Meanwhile, much of the degradation of original habitat has continued almost unabated in the Columbia River Basin and elsewhere. As reviewed by Hilborn (1992) and others, a crucial issue is the past perception that the general use of hatcheries justified habitat losses deemed necessary by societal "progress". Although over-fishing and habitat destruction are central to most serious declines in salmonid resources, recent debates have focused primarily on hatchery practices and ranching programs (Daley 1992; Martin et al. 1992).

The paradoxical question is whether the current coho salmon situation in the lower Columbia River Basin would be better off without the extensive past use of hatcheries? Presumably a more enlightened attention to hatchery programs (Brannon 1993) could have helped reduce the adverse impacts on wild stocks. However, without the past and present ranching programs, major ocean and in-river fisheries for coho salmon, along with related beneficial economic and resource uses, most likely would never have been possible. Without changing history and a significant rewrite of earlier habitat losses, including major reversals in prior readiness to restrict fisheries, lower Columbia River coho salmon without hatcheries might still today be represented by little more than relict, museum-like wild runs.

In British Columbia, and more so in Alaska, salmonid habitat is still reasonably pristine. Major ranching programs underway in these areas are thus adding to historic levels of wild-stock production. Attention is focused on a unique opportunity to simultaneously manage wild and ranched runs of salmon without undue detriment to the former. The challenge, however, is not without risk.

Although statutes and stated policies may favour protection of wild runs, wild-hatchery stock issues are complex and, until recently, were poorly understood. Well-planned experimental approaches are needed to separate inappropriate ranching practices that are detrimental to wild stocks from environmental perturbations that may impact both wild and hatchery salmonids (Walters 1988). More attention must be placed on careful monitoring of wild populations including accurate data on survival, population structures, behaviour and genetic characteristics.

Much of the basic scientific knowledge and understanding about anadromous salmonids was derived from the use of hatcheries and ocean-ranching programs. Many details of marine survival, migratory patterns, homing and straying behaviour, and other population characteristics come from the accessibility of large numbers of cultured fish for tagging programs. Preserving existing wild runs is, in part, now possible due to scientific knowledge gained from salmonid hatcheries and ocean-ranching programs.

In spite of past hatchery mistakes, salmonid culture, and ocean ranching in particular, is still an important tool with much opportunity for the continued wise use of these resources. However, the days of simply measuring hatchery success by mass or numbers of fish released are gone. Many inefficient, non-productive hatcheries along with those clearly detrimental to wild stocks should be closed. New hatcheries should be built only after careful planning and with a clear understanding they are not licenses for continued habitat losses.

Future ranching of salmonids will continue to evolve based on global resource needs and associated issues including growth of salmon farming, world markets, consumer preference, recreational fisheries, increased scientific understanding of ranched-wild fish interactions, and the capacity of oceans to produce these fishes.

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## Chapter 15

# MARKETING

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## INTRODUCTION

Success in salmon and trout farming, as in any other business activity, does not depend only on technical criteria such as the ability to grow fish successfully. In order to ensure profitability and long-term survival, businesses must devise strategies to provide them with competitive advantages over rivals (Shaw 1990*b*). Businesses that pursue excellence in marketing generally adopt strategies that are customer-driven, and that focus on customer needs and expectations. On the basis of market and competitor analysis, they strive to implement these activities to achieve maximum impact in an efficient and effective way. The purpose of this chapter is to consider the elements in this process in the context of the marketing of salmon and trout. It starts with a consideration of market analysis and major world markets. Marketing strategies are then examined looking at the elements of positioning, channel selection, business structures and promotion. Finally, the chapter reviews the progress made by salmonid producers over the past decade and considers the prospects for the future.

## THE MARKETING ENVIRONMENT

### *The Consumer Element*

Businesses operate in an external environment that is only to a limited extent controllable by their actions, but which they must monitor and analyse as part of the process of devising appropriate marketing strategies.

First, the most important element is the consumer. The demand for salmon and trout in any market will depend on a number of different factors that work together to determine the amount that will be purchased at a particular time for given trading terms. These factors can be detailed as follows:

*Eating preferences.* Diet and liking for different types and forms of food are strongly influenced by the culture of which we as individuals are a part. Seafood preferences are particularly strongly linked to our cultural backgrounds and emerge from historical traditions. In this context, the growth in farmed salmon consumption has tended to occur mainly in societies, local, regional or national, which have existing traditions of fish eating. Therefore, there is some link between overall levels of seafood consumption and preference for farmed salmon and trout. For example, consumption of farmed (and other) types of salmon is much higher in the north of Germany than in the south because the south of Germany is distant from the sea and lacks a seafood-eating tradition. Within Germany there are fewer regional differences in trout consumption. It is a freshwater fish that has been available in Germany for centuries so there are fewer barriers to familiarity to be overcome. At the same time, in many markets, lack of a strong freshwater-fish eating tradition has posed problems in the marketing of trout. This has been a barrier to be overcome in the U.K. among some consumers.

Previous experience of salmon or trout eating is not always necessary for the development of a market as is shown by the example of farmed salmon consumption in Spain. Spanish levels of seafood consumption per capita are among the highest in the world at around 25 kg per capita per annum (OECD 1988). But prior to 1980, very little salmon was consumed since it was not a native fish and there was no tradition of importing wild Pacific salmons in any volume. However by 1990, consumption had risen from virtually zero to over 10,000 tonnes as a result of the introduction of farmed salmon by Norwegian salmon marketing organisations to the market. Because of familiarity with seafood and with white fish of flesh type similar to that of salmon, Spanish consumers found it very easy to accept the new product.

At the same time, there are major differences between markets. These include differences in preferences for different types of farmed salmon and differences in attitudes toward the farmed and wild product, which are discussed further below.

*Spending power.* The availability of money to buy salmon is inevitably a factor in purchasing decisions. This is not coincidental, given its relatively high price. The major markets for farmed salmon in North America and Europe have large numbers of relatively high-income consumers. Other markets such those in Japan also have sufficient numbers of consumers with the necessary spending power. The lower prices of trout make it available to a wider section of the population. Consumer willingness to spend is not a constant and as income changes, spending changes. Bjorndal et al. (1991) found that changing levels of incomes were a major explanation of rising demand for Norwegian salmon in France during the 1980s. Even for products like the cheaper rainbow trout (*Oncorhynchus mykiss*), levels of national income have an impact. For instance, the markets for both trout and salmon were quite badly hit in the U.K. in 1990 and 1991 because of recession, largely via the effects in the food service sector as business affected by the recession reacted by reducing levels of entertainment spending. Final consumers who felt less well-off also reacted by eating less frequently in restaurants, thus depressing demand from this sector, and there were effects in the retail sector as U.K. consumers "traded-down" in their purchases.

*Information and knowledge.* A further factor affecting demand is the information which consumers have about the product — its taste, methods of preparation, and sources of supply. This is likely to be a major issue in the development of new markets when the consumer is unfamiliar with the product. Often it is helpful to introduce products first in the food service sector because consumers have an opportunity to try it without the need for cooking and preparation. Favourable eating experiences can subsequently lead to purchases in the retail sector. Norwegian generic salmon marketing was based on this approach. Knowledge about the product is unlikely to be uniform throughout a market. This is a problem in the development of demand for salmon in the British market. Only about 20% of consumers buy salmon and while consumption has been rising rapidly, it has been primarily by existing consumers eating more frequently (Gentles and Shapley 1990), so that to extend the market further there is a need for more consumer education.

*Price.* Price influences demand in any market. It is not the only influence and other conditions are equally necessary to make sales, such as good products and good distribution channels. At the same time lower prices are more likely to stimulate increased purchases than higher prices, and the evidence in the sections that follow suggest that for the markets covered, the impact of falling relative prices has stimulated substantial increases in sales (i.e., the demand for salmon is price-elastic). Falling prices in the late 1980s have brought salmon prices into closer competition with a wider range of other fish and with meats, and this has opened up new consumer segments in these markets. However, it should be noted that the responsiveness of demand to price is variable between countries and situations, and over time. For instance, while high price elasticities are reported for the U.S. market (BCSFA 1990), data from the French market suggests that demand is much less elastic. However elasticities were not constant and demand was more elastic in the second than the first part of the 1980s (Bjorndal et al. 1991). The authors suggested that this was because consumption was extending to wider income groups during the latter period. In addition, the relatively high input costs of salmon farming, particularly fish feed manufactured from expensive fish meal and fish oil, means that there is a limit to the level of price falls which can occur and leave businesses profitable (Egan 1993). This limit is likely to be above that of products like chicken with lower input costs. The over-production of farmed Atlantic salmon (*Salmo salar*) that occurred in the late 1980s is discussed further below, but it should be noted that even with substantial price reductions it was not always possible to sell all supplies and even the very substantial reductions in the price which occurred were not sufficient to solve the excess supply problem.

### *Competition*

There are many dimensions to competition. Individual producers of farmed salmon compete with other similar producers. Different types of farmed salmon compete with each other. As an example, in the U.S. market farmed Pacific and Atlantic salmon from many different sources of supply and countries compete. Farmed salmon and wild salmon compete to differing extents in different markets. The evidence suggests that farmed salmon with its consistency of product quality, supplies and greater predictability of supplies is both opening up new markets for salmon and eroding the market positions of the wild salmons with their shorter seasons and more variable quality (ASMI 1991). This is discussed further in the next section. Farmed salmon and trout also compete. For example, in Europe in 1991 the price of farmed rainbow trout fell as a consequence of the falling prices for farmed salmon. Farmed fish also compete for consumer spending with a large number of other fish, whose availability and price affect demand. For instance Bjorndal et al. (1991) reported that sea bass (Serranidae), turbot (*Scophthalmus maximus*) and brill (*S. rhombus*) are substitutes for farmed salmon in the French market. Competition with other products such as meat is also a factor. The modern consumer in affluent western societies is faced with a choice of over 5,000 items in the typical supermarket. Of course only a fraction of these are close competitors of salmonids, but ultimately all foods are competing for finite amounts of consumer spending power.

### *Marketing Channels*

The producer is helped by other businesses in marketing who provide variety of services. These include:

*Marketing Channel Functions.* These include aspects of demand forecasting, market research, production planning, quality control, packaging, transport, storage, financing, selling, order-taking, scheduling, promotion, pricing, and processing.

The economic value of these functions is reflected in the margin between farm gate prices and final retail selling prices. Occasionally the height of these prices results from the power of different stages to earn differentially higher profits (BCSFA 1990), but more typically they represent the cost of the functions to be performed to get the product to the final consumer in the form, at the time, in the place and in the volumes that they require.

A number of different types of businesses can be involved in these tasks:

*1) Wholesalers, trading companies, exporters.* Wholesalers normally buy from fish farmers or other wholesalers and their customers are retailers, the food service sector and other wholesalers. They perform a number of functions but the main function is to find trade customers nationally or internationally. They are likely to specialise in handling products from that region, which may be farmed salmon only or include a range of other local seafood items. They operate on commissions of 3-30% but these are very variable depending on functions performed.

*2) Importers, regional wholesalers.* Importers take first possession of goods sold to markets and normally sell to regional wholesalers or to large retailers, caterers and processors. In some markets importers combine regional wholesaling with their import activities or vice versa. Much will depend on the geographical distances involved.

When wholesalers are located in major market centres such as New York or Paris, a major function is to acquire products on behalf of local retail and food service customers by buying from fish farmers or other wholesalers. They are likely to be involved, in addition to selling and buying, in product transport, quality monitoring and product promotions. They usually have distribution centres for additional processing, re-packing and delivery. They operate on a commission of 8 to 30%, depending on markets and functions performed. Sometimes they process fish, particularly primary processing activities such as filleting and steaking.

In most cases such wholesalers buy a wide assortment of different seafoods to supply a portfolio of products for customers, but a wide range of types of business are to be found in practice.

*3) Brokers.* Brokers act as commission agents on behalf of producers or other wholesalers to find outlets for their products. They are used because they have special expertise in finding customers, which the producers do not possess or cannot acquire in a cost-effective way. They contact wholesalers, make sales, co-ordinate shipments, handle complaints and provide information to suppliers and wholesalers. They do not normally physically handle the product or take title to it. Brokers are a common feature of the seafood chain in North America where they take margins of 5-10% but are less common in Europe.

*4) Processors.* Salmon smokers and other businesses carrying out secondary processing usually buy directly from farmers although sometimes importers and wholesalers are used. Occasionally processors act as wholesalers when they add farmed salmon to the range of products which they produce themselves.

*5) Retailers.* Fish retailing is extremely heterogeneous with differences in different countries and different types of outlet, within the same country, but as well as between countries. Retailers range from the ubiquitous fresh fish market stall which can be found in most countries, to specialist fish retailers of various sizes with permanent facilities and to specialist fish counters, chilled display areas and frozen cabinets within large superstores. Market stall and independent specialists are usually, if not always, owner-managed businesses. Most superstores and supermarkets are usually part of multiple retailing businesses, with similar trading formats in different locations. They are often large companies, something which has implications for the marketing channel strategies of producers, which is discussed further below.

The market shares of different types of retailer vary considerably between countries. In the U.K. where retail concentration in food selling has gone furthest, multiple retailers account for 22% of the fresh fish market and over 60% of frozen fish (Keynote Report 1991). At the other extreme in Italy, multiple retailers have less than 10% of the market and most fish retailing is by independent specialists and by market stalls. There are considerable differences between the activities of large and small retailers. Large multiple retailers play much more proactive roles in transport and storage and in new product development (Shaw and Gabbott 1990). For instance in Northern Europe, fresh salmon and trout are often handled via the dedicated chilled distribution systems of the large retailers. There are also differences in purchasing. Larger retail groups are much more likely to buy fresh fish direct from the fish farmers themselves (Figure 1). Unless suitable local suppliers exist it is more usual for smaller retailers to source from wholesalers. Margins vary widely but for fresh product typically range between around 30 and 60%.

Processed products follow similar patterns. Where retail customers are large businesses the purchases will be made directly from the processor, but smaller independents are more likely to deal through processed food wholesalers.

*6) The food service sector.* There are also many different sizes of customer in this sector, but probably fewer differences in their purchasing practices. For fresh salmon and trout, even within hotel and restaurant groups, buying is usually at local level. Such purchases are usually part of an assortment of fish products purchased by that unit. They usually source products from local wholesalers who may carry out some primary processing on their behalf. Sometimes fish farmers build up their own direct links to hotel and restaurant outlets when suitable transport links exist and where distribution channels are relatively short, but this probably forms a minority of purchases. Where frozen products are concerned, larger groups have a greater tendency to buy centrally for the group as a whole because the products are less subject to variability and bulk

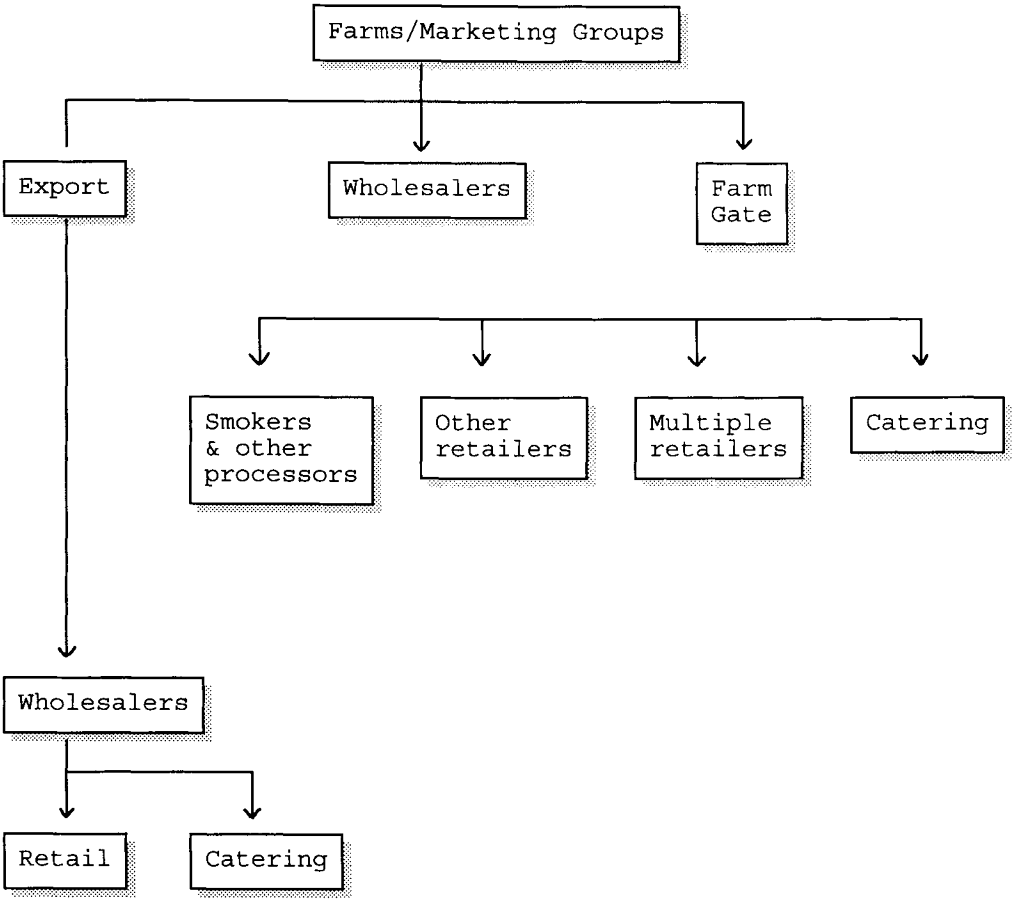

FIGURE 1. Distribution channels for Scottish farmed salmon.

discounts can be negotiated. In this situation they tend to buy directly from processors or importer/wholesalers.

Choice of channel is a key element in marketing strategy (*see* page 892). Differences in markets, customer requirements, product forms, existing trade customers, and size of salmon farmers' operations can all help to determine the appropriate channel. For example, in the U.K. there are a variety of channels (Figure 1) for farmed salmon. Some salmon is sold directly to local hotels and restaurant, but such quantities are small because most production is remote from centres of population. In the longest channels, salmon is sold first to local wholesalers who in turn sell to other wholesalers, retailers and food service outlets in both home and export markets. Salmon is also sold directly to major retail supermarket groups. After primary processing, this salmon is delivered to central drop points by the fish farmers. Retailers either deliver into stores themselves or have this done for them by specialist transport companies. Salmon is sold directly to processors who in turn use a variety of (different) channels to reach final consumers. The larger the volume of product from the producer and the larger the individual order, the shorter the channels tend to be.

Structures vary between countries. Because of retail concentration, considerable competition and relatively short distances, distribution channels in the U.K. tend to be relatively short. They are longer in the U.S. because the longer distances make it more appropriate to move product in bulk to regional distribution centres run by wholesalers rather than deliver direct into stores. At the same time there is evidence that British Columbia (B.C.) farmed salmon is opening up new distribution channels direct to large supermarkets by-passing the traditional system (Egan and Kenney 1991). On the other hand, where distances are longer, for instance in the case of Chilean exports to the U.S., exporters in Chile find it easier to sell to importers spread throughout the U.S. The importers have detailed market knowledge and are closer to the market than the Chilean exporters, justifying their intermediary role (Shaw and Gabbott 1990). Institutional factors also play a role. The Japanese distribution system (Figure 2) has long been characterised by complex and long distribution routes to the final consumer (JETRO 1990), which can best be understood by understanding their historical evolution. However, today pressure from large supermarkets is beginning to break down this structure. In general wherever retail or food service customers are large the pressure to shorten channels will exist. Farmed salmon is particularly suited to direct deliveries of this nature because of the more consistent and predictable flows of product and the ability of supplier and retailer to engage in joint planning.

### *Regulation, Exchange Rates and Other Factors*

Many other factors make up the environment within which marketing takes place but one of the most important is regulation, particularly of trade. Government regulations affect marketing in many areas from mandatory hygiene and handling standards, to regulations governing competition and trading practices. Of particular importance in recent years have been those regulations relating to external trade. It is common for most countries to impose relatively high external tariffs against imports of processed products to encourage domestic activity, thus affecting the volume of international trade which takes place, while duties on the import of raw and semi-processed products are usually lower. In addition, if importing countries consider that under the international trading rules of the General Agreement on Tariffs and Trade (GATT), trade is unfair because of the use of government subsidies or because selling prices are below production costs, additional tariffs are likely to be imposed by the

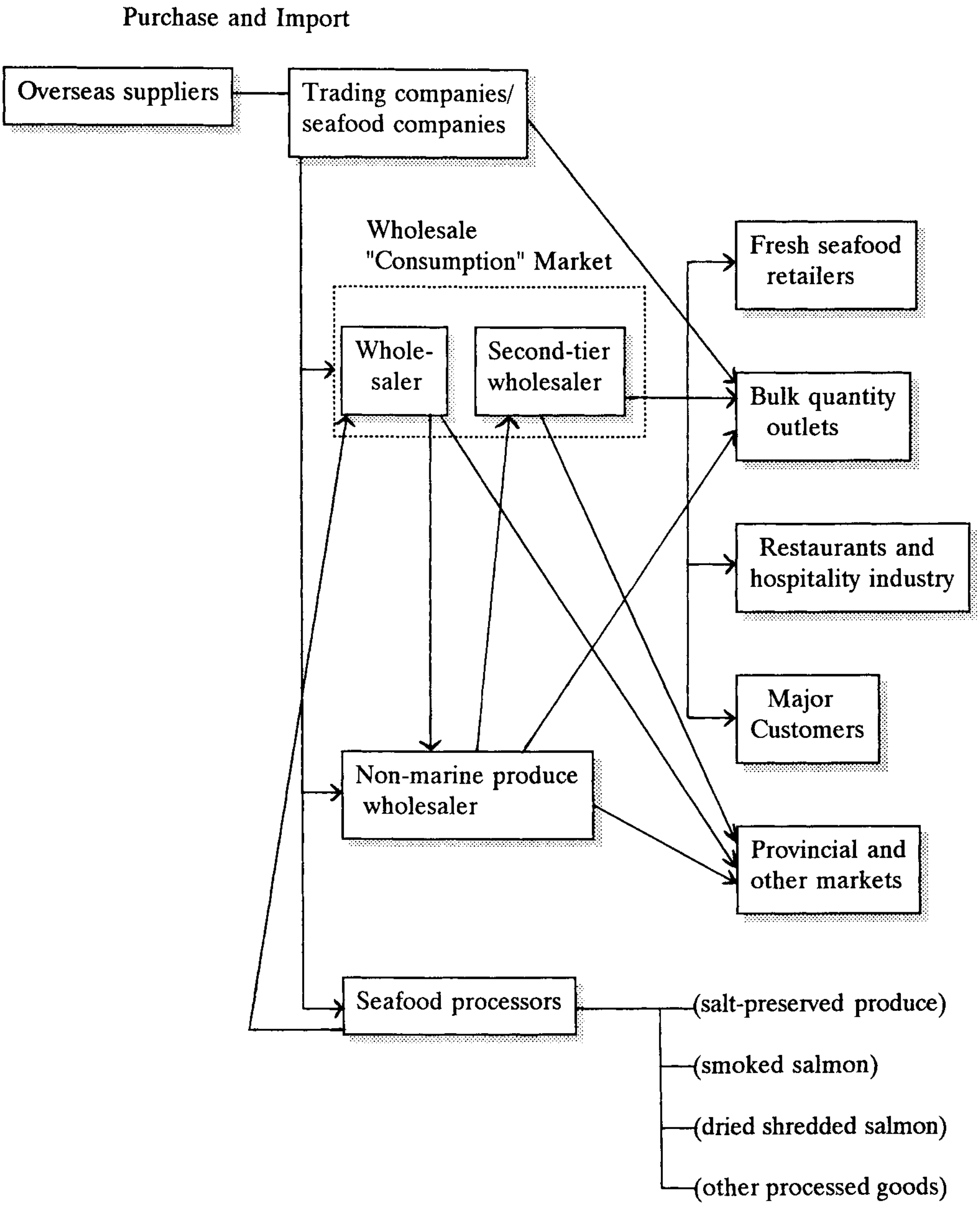

FIGURE 2. Distribution of frozen imported salmon in Japan (from JETRO 1990).

importing nation. In recent years the imposition of such tariffs has been a major issue (*see* page 885).

Trade between countries is influenced by prevailing rates of exchange which are unstable. In particular, movements of the US dollar, European currencies and the Japanese yen against each other have a major influence on the strength of demand in different markets and on the competitiveness of producers in different countries. This instability in itself can be a factor inhibiting international trade because of the risk involved in such transactions.

## MARKETS FOR SALMONIDS

### *Overview*

In most cases countries with salmon farming produce in excess of national need and export to other world markets (Tables 1 and 2). One of the most striking examples is Norwegian salmon where nearly 70% of production is exported (Table 2). Despite the large size of the domestic market, more than 60% of Scottish production is exported (industry estimates), and most Irish, Chilean, B.C., Faroese, New Zealand and Icelandic production is exported. An exception is production of farmed coho salmon (*Oncorhynchus kisutch*) in Japan which is destined almost entirely for the large domestic market (Table 1).

The major markets for farmed salmon are in Europe, North America and Japan (Table 3). Other production is exported to smaller markets in countries like Singapore, Hong Kong, Taiwan and to relatively small markets in the Middle East and South America. One of the features of farmed salmon is the variety of markets to which it is destined and the distances over which it can be transported fresh. All these markets can be supplied by air freight from any of the world's producing centres. There are small markets for salmon in many of the countries of the Middle East. Australia, supplied by its own domestic industry, while a small market at present, has considerable potential for future development. As supplies of farmed salmon have increased, efforts have been made to develop new markets, and where there is sufficient affluence together with a fish-eating tradition and non-prohibitive trading barriers the response has usually been positive.

By contrast, while farmed salmon is widely traded over considerable distances internationally, this not true of trout. Trout is produced in many western and eastern European countries, in the U.S. and Canada, in Japan and in South Africa (*see* Chapter 1) but, on the whole, most trout is consumed in the country of production. The reasons for this relate largely to demand patterns. Rainbow trout, particularly whole portion-sized fish do not command the same prices (at least under normal trading circumstances)

TABLE 1

Major producers of farmed salmon and their markets in 1990.

| Country | Output (tonnes) | Species | Major markets | Form of consumption |
|---|---|---|---|---|
| Norway | 158,000 | Atlantic | Export: Europe, U.S., Japan, Far East | Fresh, frozen, smoked, recipe dishes, marinades |
| Scotland | 40,593 | Atlantic | Domestic, Exports: rest of Europe, U.S., Japan | As for Norway |
| Chile | 17,700 | Atlantic, coho, chinook | Export: U.S., Japan | Mainly fresh and frozen |
| Japan | 25,900 | coho | Domestic | Fresh, salted, preserved |
| Canada | 21,100 | Atlantic, chinook, coho | Domestic, Export: U.S. | Mainly fresh |
| Faroes | 12,000 | Atlantic | Export: Europe, U.S. | Fresh, frozen, smoked |
| Ireland | 7,500 | Atlantic | Export: rest of Europe | Fresh, frozen |
| New Zealand | 2,200 | chinook | Export: U.S., Japan | Fresh |
| Australia | 2,700 | Atlantic | Domestic | Fresh |
| Iceland | 2,000 | Atlantic | Export: Europe | Fresh, frozen |
| U.S. | 3,900 | Atlantic, coho, chinook | Domestic | Fresh |

Source: various trade statistics

TABLE 2

Exports of Norwegian farmed salmon (tonnes).

| | 1983 | 1986 | 1988 | 1990 | 1991 |
|---|---|---|---|---|---|
| France | 4,321 | 9,904 | 18,698 | 33,679 | 29,658 |
| Germany | 2,387 | 4,671 | 7,493 | 19,204 | 12,610 |
| Denmark | 2,016 | 5,462 | 14,168 | 22,427 | 45,428 |
| Sweden | 794 | 1,916 | 12,767 | 16,773 | 13,486 |
| U.K. | 1,337 | 1,648 | 2,155 | 3,227 | 2,459 |
| Switzerland | 506 | 788 | 1,357 | 3,686 | 1,984 |
| Belgium/ Luxembourg | 484 | 1,390 | 2,137 | 8,557 | 7,752 |
| Spain | 359 | 1,917 | 3,901 | 10,333 | 12,967 |
| U.S. | 2,405 | 9,278 | 9,942 | 8,990 | 577 |
| Japan | | 771 | 2,955 | 4,657 | 4,748 |
| Others | 788 | 1,158 | 2,453 | 9,862 | 21,937 |
| Total | 15,397 | 38,903 | 68,026 | 112,299 | 126,916 |

Source: Norwegian trade statistics

that can be commanded for farmed salmon. As such, the economics of transporting the product over large distances internationally are more marginal. Further, where there is a demand for trout, this has mainly been met from local sources; freshwater sites for trout rearing are available in a wider range of geographical areas than there are waters suitable for farmed salmon production. In each market the characteristics of demand, market structures and levels of competition are different. Detailed consideration of every market is outside the scope of this chapter. As an alternative, the major markets in the U.S., Europe and Japan have been selected for further discussion.

TABLE 3

Overview of consumption of fresh and frozen farmed salmon in major world markets in 1990.

| | **Total consumption (all types excluding canned in tonnes)** | **Consumption per capita (kg)** |
|---|---|---|
| European Community | 196,234 | 0.60 |
| U.S. | 33,652 | 0.13 |
| Japan | 421,515 | 3.41 |

Source: Trade statistics, industry production statistics.

### *The U.S. Market*

People in the U.S. mainly eat fresh or frozen salmon and it is usually steaked or filleted, although there are a variety of ways in which it can be prepared from marinading to simple poaching. More salmon is consumed in the food service than in the retail sector (ASMI 1991). Some salmon is smoked using cold and hot smoking processes but this is estimated to represent a relatively small proportion of the total at around 6,000 tonnes or 3% (Anderson et al. 1990). There are differences in patterns of consumption in different areas of the U.S. On an index of 100 to represent average consumption, the consumption index in the Pacific states of 295 compares with that of 32 in the eastern-central states. Typically, those consumers have higher incomes, are more educated and eat more seafood than the rest of the population (Egan and Gislason 1989). In both the retail and restaurant sectors, about 10% of consumers were responsible for over 50% of purchases (Egan and Gislason 1989).

Table 4 shows the production, imports and consumption of fresh and frozen salmon in the U.S. market. The U.S. has major harvests of wild Pacific salmon of the chinook (*Oncorhynchus tshawytscha*), coho, sockeye (*O. nerka*), chum (*O. keta*) and pink (*O. gorbuscha*) species. Substantial quantities of this harvest, particularly pink and chum salmon, are canned and destined for major canned salmon markets in the U.S., Europe and elsewhere. Of the remainder, a considerable proportion is exported with Japan as the most important destination, especially for sockeye, although there are exports to other markets including the European Community. The other part is consumed in the U.S. market and, together with imports of fresh and frozen product, comprises the domestic

**TABLE 4**

U.S. harvest, supply and consumption of salmon, 1985 - 1989 (tonnes).

| | 1985 | 1986 | 1987 | 1988 | 1989 |
|---|---|---|---|---|---|
| Total Alaska harvest | 295,600 | 267,500 | 222,100 | 238,800 | 323,500 |
| Other U.S. harvest | 34,200 | 31,300 | 32,900 | 36,200 | 33,100 |
| Total U.S. harvest | 239,800 | 298,800 | 255,000 | 275,000 | 356,600 |
| U.S. salmon exports | 140,000 | 168,300 | 138,600 | 148,300 | 171,400 |
| U.S. pack [1] | 72,100 | 64,400 | 47,700 | 40,100 | 89,400 |
| U.S. fresh/frozen production | 96,000 | 46,400 | 54,600 | 74,600 | 69,000 |
| U.S. fresh/frozen [2] | 72,000 | 34,800 | 40,900 | 55,900 | 51,800 |
| Imports fresh/frozen [3] | 12,300 | 18,400 | 19,000 | 22,700 | 44,800 |
| Imports [4] | 11,000 | 16,600 | 16,800 | 20,500 | 40,300 |
| Fresh/frozen available for consumption | 83,000 | 51,400 | 58,000 | 76,400 | 92,100 |
| January 1 inventories [5] | 18,700 | 27,300 | 19,100 | 95,700 | 20,500 |

[1] Canned weight

[2] Adjusted for recovery at 75%

[3] Product weight

[4] Adjusted for recovery at 90%

[5] Cold storage

Source: Alaska Seafood Marketing Institute, Salmon 2000.

market for fresh and frozen salmon. It is into this sector that farmed salmon is supplied, mainly as fresh product.

Within this sector, the share of farmed salmon grew in the 1980s. The U.S. has its own farmed industry, most notably for Atlantic salmon on the Eastern Seaboard. This produced 4000 tonnes in 1990, mainly of Atlantic salmon and destined for the domestic market. However, the main source of supply for that market is imports of fresh farmed salmon. Estimation is difficult because farmed salmon cannot be separately identified but it is believed that most imported fresh salmon is, in fact, farmed, with the exception of some of the imports coming from Canada. ASMI (1991) estimated that farmed imports amounted to 17% of total 1989 supplies for the fresh and frozen market. These imports came primarily from Norway but there were also imports of farmed Atlantic salmon from Canada, Chile, Ireland and the U.K. Farmed Pacific salmon is imported from Chile, Canada and New Zealand. These imports grew steadily during the 1980s (Table 5), but in 1991 the balance of supplies changed considerably as a result of a successful anti-dumping action against Norwegian salmon exports. The additional levies as high as 26%, which were imposed against Norwegian imports, combined with appreciation of the U.S. dollar against European currencies has severely curbed imports from Norway and other European producers. The main beneficiaries of this change were Canada and Chile (Egan 1991).

The U.S. market for fresh salmon has been growing and is speculated to have considerable potential for further development. Within this, the potential for the further development of the market for a farmed product is considered good (Egan 1991) since most salmon consumption is presently confined to a small section of the population. Egan (1991) concluded that a key factor in the potential for growth of consumption is the year-round availability offered by the farmed, but not the wild product, as the latter is only available for about five months of the year. At the moment consumers are confused about the differences between wild and farmed salmon, but they do have a strong preference for fresh rather than frozen salmon. This gives farmed salmon an advantage because it is difficult to supply wild Pacific salmon fresh year round. There is also some potential for smoking farmed salmon since, at present, the market for smoked salmon in the U.S. is very small in comparison with that in Europe.

Egan's (1991) study for the Canadian Department of Fisheries and Oceans (CDFO) also concluded that the demand for salmon is very sensitive to price changes. This is a major issue to be addressed in the future, given the limited scope for further price reductions consistent with viability of production. Consumer education, improved product quality and availability appear to be more fruitful marketing approaches. It is particularly important that effort is concentrated on the retail sector where sales of farmed salmon are relatively undeveloped in comparison with the restaurant sector.

Markets for trout in the U.S. are less complex and smaller. There are markets for trout throughout the U.S., with greatest consumption on the east and west coasts. Trout

**TABLE 5**

U.S. fresh salmon imports (tonnes).

| Origin | 1987 | 1988 | 1989 | 1990 | 1991 |
|---|---|---|---|---|---|
| Canada | 1,620 | 6,000 | 11,470 | 13,013 | 21,530 |
| Chile | 834 | 1,175 | 2,062 | 7,058 | 11,056 |
| Norway | 7,640 | 8,930 | 11,538 | 7,699 | 575 |
| Other | 1,317 | 1,306 | 2,727 | 1,982 | 3,025 |
| TOTAL | 11,408 | 17,411 | 27,768 | 29,752 | 36,186 |

Source: U.S. trade statistics.

are sold dressed and gutted usually at a weight of around 250 to 450 g and are usually white fleshed, although in recent years there has been some growth of production of fillets and boned products. There is also a limited availability of value-added products such as breaded items. Both fresh and frozen products are marketed but the most rapid growth in recent years has been in the fresh market.

The trout market is supplied almost entirely from domestic sources. Production is in the 25-30,000 tonne range (Table 6). The largest concentration of trout production is in Idaho where there are three large producers including Clear Springs Foods who alone produce around 10,000 tonnes per year. The ideal growing conditions and low production costs of the large scale Idaho operations enable them to supply markets throughout the U.S. with their own refrigerated distribution systems (Wray 1988). Other producers can be found throughout the U.S. but they supply local rather than national markets.

The trout market has grown steadily but not spectacularly over the past 15 years. Profitability has been adequate but not sufficient to lead to rapid growth of production. In any case the high cost of developing new sites including the cost of meeting new environmental controls, lack of water, and thus lack of suitable low cost sites has precluded much expansion. This has led to general stability in the industry and little significant change is expected in the future. Competition from farmed salmon is a potential problem but trout markets are somewhat specialist and production is relatively small in relation to the total volume of salmon available. The trout market has not been significantly affected by large salmon harvests in the past so that major adverse impacts of future increases in salmon supplies are not expected.

**TABLE 6**

The U.S. market for rainbow trout (tonnes).

| | 1987 | 1988 |
|---|---|---|
| Domestic production | 25,933 | 27,279 |
| Imports (all types) | 918 | 635 |
| Exports | — | — |

Source: FAO, U.S. trade statistics.

### *European Markets*

The European Community is a major consumer of salmon, particularly farmed Atlantic salmon, which is preferred to the Pacific salmons. It is estimated that around 170,000 tonnes were consumed in 1991. Despite free trade between the member states of the European Community and close trading links between countries, each European Community market is different, particularly in terms of the levels of consumption. France is the largest salmon market in Europe, with the highest per capita consumption level and probably the widest consumption throughout the population as a whole. The other substantial markets are in Germany, the U.K., the Netherlands and Spain. During the early 1980s consumption was considerably greater in northern than in southern Europe because these were traditional salmon-consuming markets and because they were the main target of marketing by producers, particularly the Norwegians. One of the most important changes over the last five years has been the growth of consumption in southern Europe notably in Spain and northern Italy, which responded rapidly to marketing efforts and who are still considered to have good future growth potential. There are some differences in product preferences between markets but one of the most striking features of the European market is the importance of salmon smoking. Most of this salmon is smoked within the Community, including the salmon which is imported from Norway. There are substantial salmon smoking industries in the U.K., France, Germany, Belgium and the Netherlands, and salmon smoking is growing in Italy and Spain. No precise statistics are available but it is estimated that in France over 60% of salmon is consumed in smoked form, almost entirely by cold-smoking processes. Smoked salmon is a major outlet for farmed salmon in all Community countries. Smoked salmon is also exported from the Community to other countries.

Most of the rest of the salmon is consumed fresh, although frozen salmon is also available. Typically fresh salmon is sold by specialist fish retailers or by the wet fish counters of supermarkets where sellers will steak or fillet the fish for customers as required. There has also been a great deal of product innovation. Steaks and fillets are available in vacuum packs and in controlled atmosphere packs, and at least one major British retailer has dispensed with wet fish counters to sell all fish products in this form. A wide variety of recipe dishes are available such as salmon in pastry and salmon goujons. Marinaded salmon is also popular. The issues involved in these types of value-added activities are discussed later in this chapter (*see* page 892), but this does mean that the European market is a complex one and forecasting and assessing demand therefore involves the analysis of a number of different sectors, all with different characteristics. The market for farmed salmon is year-round, although sales of the smoked product peak strongly in the last two months of the year.

Farmed salmon is supplied from three main sources: Norway, the U.K. (Scotland) and Ireland. Norway dominates with over 40% of supplies. The European Community is Norway's largest market, taking nearly 80% of exports in 1990 (Tables 1 and 2). Norway is not itself currently a member of the Community and therefore liable to restrictions on entry to the Community's markets. It is liable for a 3% entry duty on fresh and frozen salmon and a reference price system is also currently in force, albeit probably on a temporary basis (Redmayne 1992). The Community, like most major trading areas, imposes higher duties on processed than on fresh product so that imports of processed salmon from Norway carry a duty of 15%. There are other minor outside suppliers such as the Faroe Islands and Iceland. France and Spain are also minor producers. There is little farmed Atlantic salmon imported from other areas; the attractions of the North American market and comparative costs deter exports of North American salmon and farmed salmon from other sources. Chile and New Zealand have so far found it more profitable to export to the North American market, to Japan and to the Far East, although increased exports from Chile to European Community countries can be expected.

The European Community also consumes wild salmon. While the quantities of wild Atlantic salmon available are very limited, the Community imports all species of wild Pacific salmon. The rise of European salmon farming has had a major impact on this situation. Almost all the growth in the European market, which occurred in the 1970s and 1980s, has been due to increasing consumption of farmed Atlantic salmon. The latter has a competitive advantage on the grounds of availability and the short order times involved. For instance fresh salmon can be delivered by container lorry from growing areas of Norway to the major markets in Paris within 24 hours. Although there are minor specialist markets for high-value wild Pacific salmon such as king (chinook), when wild Pacific salmon is imported today it is on price grounds. For this reason chum and pink salmon are still imported on some scale and there are signs that the falling wild Pacific salmon prices associated with larger landings in recent years have led to some recent increase in imports. These low-priced and often low-quality products are usually destined for smoking.

The rapid growth of consumption in the Community reflects a number of factors. As indicated earlier, as an affluent market with fish-eating traditions it was an obvious target for producers. Salmon was known especially in northern Europe and had a highly favourable consumer image. There has been a general trend toward the consumption of lighter meats and fish in many areas of Europe, so again, salmon was provided with a market opportunity (BCSFA 1990). The proximity of the European market to the European producers was also a major consideration, as were the relatively high prices of white fish due to scarcity of supplies. For this reason further growth is expected in demand in the future, particularly in markets such as the U.K., Spain and Italy, which are considered to be relatively underdeveloped at present.

More than 150,000 tonnes of rainbow trout are consumed annually in the European Community and the market has grown substantially over the past decade, almost entirely due to growth in domestic production. Trout are currently sold in size ranges between 200 and 1,000 g but the 250-300-g size is most popular. Trout are sold live, round, or gutted and filleted, both fresh and frozen. Trout fillets are becoming increasingly popular and are usually prepared from 280-g fish. In 1989/90 fillets accounted for 20% of sales in Italy, 10% in Denmark and France, and smaller percentages in other countries (Gabriel 1990). Flesh colour varies from country to country and depends on end use. In the U.K., Italy and Ireland, pink-fleshed trout account for a significant proportion of portion-sized consumption, while in France most portion-sized trout is white flesh. For fillets, generally pink-fleshed products are preferred. Probably around 10% of trout is frozen and most of the rest is sold fresh. Sales are year round. There is some smoking of trout, particularly in Germany where it probably accounts for up to a third of total consumption, although quantities in other countries are very much smaller than this. There is a limited amount of other processing in prepared dishes, (e.g., patés) but, while there is a considerable product range, value-added products in total account for only a small proportion of total consumption.

The largest markets are in Germany, France, Italy and the U.K. while the largest producing countries are France, Italy and Denmark. Rainbow trout are produced in every member state of the Community (Table 7) and there are major industries in the U.K., France, Spain, Germany and Denmark. Only 2-3% of supplies are sourced from outside the Community. Imports of portion-sized trout come primarily from eastern Europe and imports of large trout are from Norway and Finland, but volumes of intra-Community trade in trout are larger (Table 8). Germany is a major importer of trout, mainly from Denmark but also from France and Italy. France also imports from Denmark and from a number of other countries as do Belgium and the Netherlands.

During the later 1970s and early 1980s real prices declined and many businesses showed losses. The state of the industry improved during the latter part of the 1980s because real selling prices stabilised as a result of stronger demand, while input costs fell, thus improving profitability (Gabriel 1990). This successful phase was halted recently because the decline in salmon prices and economic recession have caused a fall in trout prices. The industry is also liable to instability and cycles in profitability because of difficulties of forecasting demand over the production cycle and the very large number

TABLE 7

Production and consumption (tonnes) of trout in Europe in 1989.

| Country | Production | Imports | Exports | Consumption | Per capita (g) |
|---|---|---|---|---|---|
| Belgium | 800 | 5,56 | 1,109 | 4,947 | 501 |
| Denmark | 30,500 | 480 | 23,195 | 7,785 | 123 |
| France | 33,000 | 3,914 | 4,284 | 32,630 | 589 |
| Germany | 15,500 | 20,500 | 238 | 35,762 | 584 |
| Greece | 1,800 | 7 | 4 | 1,803 | 180 |
| Ireland | 1,200 | 179 | 206 | 1,173 | 331 |
| Italy | 32,000 | 505 | 2,281 | 30,224 | 530 |
| Netherlands | 100 | 1,903 | 217 | 1,886 | 129 |
| Portugal | 1,165 | 2 | 1 | 1,166 | 113 |
| Spain | 16,850 | 292 | 438 | 16,704 | 431 |
| U.K. | 16,500 | 2,067 | 608 | 17,959 | 316 |
| Total EC | 149,415 | 35,105 | 32,581 | 152,039 | |

Source: Eurostat annual trade statistics, unpublished proceedings of FES Annual Assembly, 1990.

of producers involved. However, it is expected that prices will improve because of the generally buoyant demand for farmed fish products in Europe, coupled with scarcities leading to high costs of wild fish (Shaw and Bailly 1990); this already appeared to be happening in early 1992. Future prospects are only for slow growth, partly because of supply constraints. Growth is constrained by a shortage of suitable sites and the implementation of more stringent environmental standards in Europe, although there are some sites available in some areas such as Scotland, Spain and France.

### *The Japanese Market*

In absolute size and in terms of per capita consumption, this is the largest salmon and trout market in the world. Japan is one of the world's largest markets for all seafood and the demand for salmon and trout is a part of this.

Japanese consume salmon and trout mainly in the form of fish fillets prepared for grilling (kirimi). This used to be salted but in recent years tastes have shifted from salty

**TABLE 8**

Intra- and extra-European Community trade in trout in 1990 (tonnes).

| | | % production |
|---|---|---|
| Production | 155,365 | - |
| Intra-EC trade | 29,105 | 19 |
| Exports from EC | 4,209 | 3 |
| Imports from EC | 4,929 | 3 |

Source: Federation European de la Salmoniculture, Eurostat.

to sweeter fish and from frozen to fresh fish. Some salmon is smoked, but at around 10,000 tonnes (JETRO 1990) this represents a relatively small proportion of the total market. Salmon and trout are used in a variety of other dishes such as pickled dishes, and are served dried and shredded. In recent years they have also been eaten raw in sushi and sashimi dishes. More salmon and trout are consumed during the period from July to December, but this mainly reflects the pattern of existing supplies and there are no real barriers to year-round consumption when product is available.

The market for salmon developed during the 1950s and 1960s and most supplies originally came from the catches of the Japanese high seas fleet. With the North Pacific Fisheries Agreement which led to a reduction in this fleet, the U.S. and Canada became more important sources of supply of wild Pacific salmon. Meanwhile, the Japanese developed the ranching of chum salmon with landings that rose from less than 58,000 tonnes in 1985 to the present steady state of around 185,000 tonnes (JETRO 1990). In the later 1980s coho salmon farming also developed in Japan and in recent years, farmed salmon imports, especially from Norway and Chile, have been rising.

The total market for salmon of all types (excluding canning) amounts to about 420,000 tonnes with supplies dominated by wild Pacific salmons and ranched chum. Imports of frozen salmon, mainly sockeye, amounted to 38% of consumption in 1990 and salmon from the Japanese high seas fishery amounted a further 8% of consumption. Ranched chum salmon represents 48% of the total (*see* Chapter 14). Farmed salmon is supplied from Japan's own coho production, which amounted to around 21,000 tonnes in 1990. In addition farmed Atlantic salmon are imported mainly from Norway and coho salmon are imported from Chile. Together, these farmed salmons represent only around 7% of total supplies.

The total salmon market has been growing in recent years, but this is largely because it is very price sensitive and growth has been a response to recent price reductions. Given the existing high levels of consumption it is also likely that there is little scope for further non-price led growth in the total market size. Salmon faces strong competition from beef and from other fish products, and there is an expectation that if world beef prices fall, this will have an adverse effect on fish consumption of all types (JETRO 1990). In general there has been a trend, unlike the U.S. and Europe, toward increasing red meat and chicken consumption and away from seafood (ASMI 1991).

The relatively slow development of farmed salmon consumption, certainly in comparison with Europe, is linked to existing preferences and patterns of consumption. There is a preference for bright red salmon such as sockeye, which command high prices in the Japanese market. It has been difficult to build demand for the more delicately flavoured and coloured farmed salmon and prices are considerably below those paid for wild sockeye salmon. However, there has been much greater interest in recent years. The high quality, consistency and year-round availability of farmed Atlantic salmon from Norway has created markets. Norwegian salmon is popular for use in salmon steaks in the food service sector where it is attractive because it is a product which is not normally served at home (ASMI 1991), giving restaurants scope for product differentiation. It is also supplied to supermarkets and used by the domestic smoking industry. Farmed salmon from Chile has also been very successful, which no doubt owes something to the close trading and ownership links between the Chilean industry and Japanese seafood businesses, with investments by Nichiro and Nihon Suisan in farming businesses in Chile. Good transport links for frozen farmed coho have been developed between the main Chilean growing areas and Japan. In general imported farmed salmons achieved much higher prices than domestic farmed coho, which have a relatively poor reputation (ASMI 1991). Thus within the overall static total, there is likely to be further scope for increased consumption of farmed salmon, but it appears that price will continue to be a major issue in choice.

Markets for trout are smaller. Supplies are dominated by imports, which come primarily from the U.S. and are of portion-sized freshwater product (Table 9). Domestic production of trout has been declining due to problems of rising costs and low profitability. A recent trend has been a growth in imports of larger fish 2 kg and over that have been reared in seawater. These imports come from Sweden and Norway and are used in sushi, sashimi and for grilling and light salting, and compete most closely with farmed salmons.

## MARKETING STRATEGIES FOR SALMONID PRODUCERS

### *The Key Decisions*

There are a number of strategic options open to producers. The key decisions are concerned with the markets to be selected, the types of products to be produced and the

**TABLE 9**

The Japanese trout market (tonnes).

| | 1987 | 1988 |
|---|---|---|
| Domestic production | 19,735 | 16,000 |
| Total imports | 110,681 | 133,613 |

Source: JETRO (1990)

types of channels to be used to reach those markets. These decisions are related to the nature of the business in that certain strategies may require certain types and sizes of business organisation. After these decisions are made, decisions on how to handle product management, selling and promotion in the chosen markets can be made and implemented. This section is concerned with key strategies, while the next is concerned with implementation.

Each market has different requirements in terms of product specifications, product volumes, quality, and sizes. Thus a key choice is the selection of markets, in particular the choice between home and export markets, the latter typically requiring higher levels of expertise in trading. Next, within markets there are different market segments, each with different requirements. Often these differences are related to the type of marketing channel selected for the product. Channels often have specific product requirements that are unique to that channel, and the structure and operation of that channel also leads to specific requirements, such as for handling and transportation. For example, in the U.K. each supermarket group has different product specifications and handling arrangements and the form in which they buy the product (e.g. whole, filleted, steaked) varies. Ordering and supply requirements and volumes required may be different from those required by wholesalers.

Decisions to produce more highly processed products lead to additional requirements. Markets for more highly processed products serve different consumers, may involve different marketing channels and involve major changes to business activities.

Producers must identify clearly the particular market segments or group of segments that they wish to supply. Failure to do this and to relate activities to the needs of those segments is likely to mean loss of sales to other competitors who relate their activities more closely to these needs. These choices must be made in light of the capabilities of the businesses. There is no point in carrying out strategies that leave the business at a competitive disadvantage in comparison to other competitors supplying the same market. The key is to select the strategies which are the best fit, or, having chosen a particular strategy, to make necessary changes in organisation to ensure that this is the case.

### *The Size Factor*

Because decisions on strategy are company-specific and each company has actual or potential strengths that dictate particular choices of strategy, there is no single model. These differences have led to the emergence of a number of different patterns although there are some common tendencies across countries. One of the most important is the role of size in competitive success. For salmon, as a generalisation, those producers who are relatively large (i.e., selling at least 3,000 tonnes of product) have both more options than their smaller counterparts and are better placed to achieve strong competitive positions. Size in marketing conveys a number of advantages (Shaw 1988, 1990*a*), namely:

(1) Access to large customers. Larger growers can supply larger customers year-round at no cost penalty because of their command of greater volumes. This is particularly important where the multiple retail sector has a substantial proportion of sales and wants to deal directly with growers themselves (e.g., U.K., Belgium, France);

(2) Risk. Larger growers are more likely to be able to supply a spread of markets and customers cost effectively, thus reducing risk since the loss of a single account becomes less critical; and

(3) Expertise. There are substantial set-up costs associated with running an effective marketing operation staffed with qualified personnel and able to undertake necessary market research, sales visits and other forms of promotion. Having setup such an operation, the incremental costs of handling additional volumes are low.

Finally, when a large number of small producers are trying to sell to the same trade customers, their bargaining power is weak. In the early years of the development of the B.C. salmon farming industry this is considered to have depressed prices received ex-farm (BCSFA 1990).

Research on salmon marketing in Scotland (Shaw 1988) suggests that larger suppliers are more likely to export, able to meet its demands for greater marketing expertise, and more likely to deal directly with large retailers and processors than are small companies. They are also, in some cases, supplying a wider range of outlets, helping to reduce risk. Smaller producers are excluded from attractive and important markets because they cannot supply volumes required. They cannot supply customers continuously throughout the year, which makes it more difficult to build loyalty (Table

10). They are required to make use of longer marketing channels, which reduce their margins.

As the Scottish salmon industry has grown and as competitive pressures have become greater, so there has been a general tendency for smaller producers to adopt forms of marketing organisation which can achieve similar economies to those of larger companies. Development of these options together with merger and take-over, changed the marketing situation in Scotland from one where in 1988 there were 90 selling points selling 12,000 tonnes of salmon to the situation in 1990 where 7-8 selling points controlled 80% of sales of 33,000 tonnes of salmon (Shaw 1990). Further rationalisation is expected and similar trends are occurring in other countries.

Smaller producers can re-organise in a variety of ways to offset size disadvantages (Table 11). In Scotland all of these solutions can be found. Some producers grow under contract to larger companies such as Marine Harvest, thus allowing themselves to concentrate on production, leaving marketing to the larger and more experienced company. Others have close relationships with local wholesalers with specialist marketing expertise such as Framgord in the Shetland Islands who have a packing station and sell to markets worldwide (Scottish Fish Farmer 1991). Some producers sell through joint marketing companies of which Aquascot is an example. This marketing and company is owned by 12 fish-farmer shareholders and a further 15 farmers sell to them. It operates a primary processing plant and sells to a variety of different international markets as well as to the domestic market (Fish International 1990).

Joint initiatives such as this are not easy to set up and manage. Growers have to be willing to allow the marketing arm of operations to dictate to the rest of the business and they also have to be willing to forego their independence. A major problem in such groups is gaining the necessary commitment from all members to work in this way (Shaw 1990). Nevertheless, these examples of success indicate that this can be a viable option. It is also an attractive option for growers who wish to remain independent of major international companies but who wish to be able to compete for the major markets which demand volume, consistency and quality (Fish Farming International 1991).

In Norway, there is a legal constraint on the size of each production operation of around 300 tonnes. Until recently all sales by these units had to be made to licensed export organisations of which 15 commanded 80% of sales, with three or four of these companies sharing 50% of total sales (Shaw and Gabbott 1990). It was in this way that the benefits of size in marketing were achieved.

Similar trends are observed as the industry develops in British Columbia, Canada. As competitive pressures have increased, so farmed salmon producers have found it increasingly difficult to survive outside larger marketing arrangements.

TABLE 10

Seasonality of sales of growers of different sizes in Scotland, 1988 (% of growers in each size category supplying each month)[1].

| | Production size (tonnes) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1-10** | **11-50** | **51-100** | **101-300** | **301-500** | **501-1000** | **1000+** |
| No. of companies in size category | (2) | (7) | (1) | (5) | (2) | (5) | (5) |
| January | 0 | 57 | 0 | 80 | 0 | 100 | 100 |
| February | 50 | 43 | 0 | 40 | 0 | 100 | 100 |
| March | 50 | 71 | 0 | 80 | 100 | 100 | 100 |
| April | 50 | 86 | 0 | 40 | 100 | 100 | 100 |
| May | 50 | 71 | 0 | 40 | 50 | 75 | 100 |
| June | 100 | 86 | 0 | 40 | 100 | 75 | 100 |
| July | 100 | 43 | 0 | 60 | 100 | 100 | 100 |
| August | 100 | 71 | 100 | 80 | 100 | 100 | 100 |
| September | 50 | 43 | 100 | 80 | 100 | 100 | 100 |
| October | 50 | 57 | 100 | 100 | 100 | 100 | 100 |
| November | 0 | 71 | 100 | 100 | 100 | 100 | 100 |
| December | 0 | 57 | 100 | 80 | 100 | 100 | 100 |

[1] These percentages do not add up to 100 because growers often sell salmon in several months.

Source: Shaw (1988)

TABLE 11

Some marketing options for smaller growers.

| Option | Merits | Problems | Key success requirements |
|---|---|---|---|
| 1. Contract sales to larger sales. | - Little marketing effort required.<br>- Little finance involved. | - Little control.<br>- Risk of breakdown of agreement in weak markets. | - Clear specification of rules of relationship including output committments, financial, arbitration, pricing procedures. |
| | - Access to marketing and technical skills of larger growers. | - Large growers may not co-operate. | - Avoid long-term price agreements.<br>- More power if organised via a grouping of smaller growers. |
| 2. Formation of marketing company other growers (via share ownership). | - Control of large production volumes gives marketing economies of scale and access to all markets.<br>- Ability to buy marketing expertise.<br>- Entry into primary and secondary processing possible. | - Achieving harmonious working of company.<br>- Some loss of individual control.<br>- Conflicts if share holdings not in proportion to output. | - Large volumes of output (at least 3,000 tonnes and more with long-term).<br>- Equal shareholdings of members.<br>- Clear written agreements, specifying detailed obligations of all parties, strong discipline, arbitration procedures.<br>- Committment to allow company to be marketing-led, buying from farmers at realistic prices and according to market needs.<br>- Participation of growers with similar interests (e.g., similar size). |

**Table 11** cont'd.

| Option | Merits | Problems | Key success requirements |
|---|---|---|---|
| 3. Formation of co-operative. | - as in 2. | - as in 2. | - as in 2. However possible difficulties in raising finance (depends on national law for co-operatives). Currently less popular in U.K. Need for restrictions on membership to ensure commitment. |
| 4. Joint company: growers and wholesalers/processors. | - as in 2.<br>- Easy acquisition of marketing expertise. | - as in 2. | - as in 2.<br>- Agreement of wholesaler only to market output of members of group.<br>- Choice of wholesaler with high levels of marketing expertise. |

The situation for trout producers has many parallels but is not exactly analogous. There are many small and successful trout producers in this relatively mature industry. In the European Community, 93% of enterprises produce less than 200 tonnes and they account for over 50% of production. Within the Community in Germany, there are 4,000 producers, most of whom are family-based operations employing no staff (Gabriel 1990). Unlike the salmon industry, many have not found it necessary to link with other organisations for marketing. They supply local markets selling directly to retailers, the food service sector or to final consumers via farm shops and delivery services. They can do this because they are located closer to centres of population, unlike the salmon producers who are usually found in more remote locations. By selling directly further down the channel, they can achieve higher ex-farm prices than if selling through established wholesale systems. Klontz (1991), citing North American experience, suggests that there are many opportunities for small trout producers to sell bone-out fillets and specialist smoked salmon products to niche markets. Often these small producers have comparatively high production costs but they can co-exist with larger lower cost producers because of the different markets and channels used. In Germany for instance, most of the 16,000 tonnes of trout produced is marketed very close to the farms, which can be found throughout the country, and often some of this trout is smoked before sale.

This option is not available for all small trout producers because of limitations in the size of local markets, similar to those faced by salmon farmers. For those whose output has to be destined for larger national and regional markets, the same size arguments apply to businesses in the trout sector as in the salmon industry (Fish Trader 1992). In Germany, while local trout farms supply local markets, national distribution systems use trout imported from large low cost producers in Denmark and Italy (Lasch 1989). Similarly in the U.S., the low costs of trout production in Idaho, the main producing centre, gives them a competitive advantage which enables them to supply markets throughout the country.

Within this framework, there are still a number of choices to be made by producers. For instance, in Scotland, even among the larger companies, there are differences in strategies. The largest company, Marine Harvest, has chosen a relatively wide range of outlets including moves forward into processing (discussed further below). Other companies have concentrated on a more limited number of accounts, perhaps building up particularly strong relationships with processors. McConnell Salmon is linked to a fish processor, Bluecrest, owned by the same company, Booker McConnell. Others have kept out of processing but have built up close relationships with large processors. They have selected different markets, and the percentage of output exported is different in different companies. Often the explanations are historical. Relationships developed during the early stages of the industry have been maintained and individual accounts have grown with the size of markets. Expertise built up in the supply of particular customers with precise product quality specifications and logistical requirements enables that business to be retained over time. Strategies for markets are also greatly influenced by decisions about developing down the value chain to the consumer.

### *Processing and Added Value*

Thus far the analysis has assumed that the main choices open to producers are concerned with strategies to market fresh product, but we have seen that salmon and trout can be processed in various forms. Marks and Spencer's salmon products shown in Table 12 give an indication of the range of product forms available. This adds value to the product which can potentially increase the total profit of the business (Table 12). By creating more highly differentiated products it is also a means of creating distance from competitors and building customer loyalty. It can be a way of reducing risk, both because it secures customers further down the value chain and because it can increase the range of markets supplied. However, like other strategic choices, the balance of arguments should be considered before deciding whether this is a sensible option for the business. There are many potential hidden costs and risks in vertical integration. Options should be considered in relation to the alternative, which is rather than competing with processors, having them as independent customers.

The first issue is the nature of the opportunity. Processing is only one outlet for salmon and trout, and in some countries this is a relatively small market. Hence, processing options have not been a major consideration by B.C. salmon farmers since the major opportunity lies in the sale of fresh salmon to the Canadian and U.S. markets. In comparison with Europe, markets for smoked salmon and for other processed salmon products are relatively small at present.

Secondly, the international food processing business is sophisticated and efficient and to succeed, the salmon or trout farmer must add to their skills in growing the rather different skills of food processing and marketing of processed products. It usually requires considerable investment and large-scale operations, as economies of scale are more significant in food processing than in salmon farming (Shaw 1990). There may also be a need to process a range of products other than salmon and trout in order to build the wide portfolio which may be demanded by some customers. Management must be able to acquire the necessary skills and scales of operations to compete effectively with specialist, non-integrated processing businesses. This is particularly true of the fast growing area of chilled recipe dishes. Many developments with salmon and trout recipe products have come not from integrated farming and processing operations, but from companies who specialise in the production of chilled prepared products. The latter achieve economies of scope across many lines with many ingredients of which salmon is only one. They are also able to focus product development work on lines (e.g., pasta dishes, pastry dishes) with a variety of different flavourings rather than being limited to one ingredient. In this way product development work can be more cost effective.

Another barrier to forward integration can be regulatory. While Norway has a major salmon farming industry, it has only a small processing industry. A substantial proportion of its output is destined for smoking by processors in the European Community who in turn supply both their domestic and export markets. There are two

TABLE 12

Marks & Spencer's salmon product range and prices in the U.K., August 1992.

| | Fish weight (g) | Unit price (U.S.%) | Price per kg (US$) |
|---|---|---|---|
| Salmon Cumberland pie | 225 | 3.78 | 16.63 |
| Salmon à la crème | 225 | 3.78 | 16.63 |
| Salmon parcels filled with mushrooms and cream | 250 | 7.58 | 30.32 |
| Salmon escalopes | 200 | 7.13 | 35.65 |
| Two salmon fillets and Hollandaise sauce | 283 | 9.48 | 33.18 |
| Six salmon fillets | 750 | 14.25 | 18.52 |
| Salmon kebabs | 280 | 9.48 | 33.84 |
| Salmon steaks | 250 | 6.75 | 27.00 |
| Caledonian fillets | 250 | 8.84 | 35.36 |
| Scottish smoked salmon | 113 | 7.13 | 59.89 |
| | 454 | 22.78 | 50.12 |
| Smoked salmon parcels | 124 | 5.68 | 46.00 |
| Poached salmon terrine | 113 | 3.40 | 30.06 |
| Gravalax | 113 | 5.68 | 50.21 |

explanations for this. The first relates to the strength of competition from long-established smokers in France, the Netherlands, Denmark and Germany who have particular expertise in understanding the specific requirements of their domestic markets. The second explanation is the duty barrier of 15% faced by processed exports from Norway into the European Community, making processing activities in Norway uncompetitive.

There is a danger in integration, unless objectives and rules are set very carefully, that the acquisition of a new type of business is a diversion from core activities. This can be inefficient because there is a danger that by trying to combine the needs of two different operations, neither can operate optimally. Both would perform better if free to source or supply customers where they choose. For this reason, those businesses that are integrated through common ownership are often given the freedom to source or supply as they choose and are managed in a very decentralised way. A further issue is the question of competitiveness. Forward integration means facing new types of competitors in an area where the business may not have existing expertise, which can make it a weak competitor.

There is once again, no single structure which is optimal. Some companies do have the necessary managerial skills and resources and have chosen to expand through vertical integration, while others have concentrated on their core business of growing. Where major markets are targeted, the companies following this route tend to be large or part of large groups, once again because of the advantages of size for competitiveness. However, much would appear to depend on the other business interests of such companies. Those who have moved furthest tend to be ones where, within the parent group, there is food processing knowledge and expertise. There are smaller businesses who have followed this route. In these cases, they tend to target on high quality niche markets where there is product differentiation and where low processing costs are not the key issue in selling.

The types of business structure to be found are the following:

*Non-integrated.* These are businesses that sell only fresh whole salmon or trout and who have no interests in downstream activities.

*Farmer/primary Processor.* These are growers who have extended their activities to primary processing (i.e., they clean, gut, head and fillet fish). This is a growing trend, associated with consumer demand for higher quality products and the raising of hygiene standards by governments. Often because of the size of necessary investment in processing plants, this has accelerated the combination of smaller producers into marketing companies who build plants for this purpose. There are large companies in this category. As an example, Clear Springs Foods, Idaho, the largest trout producer and processor in the world, is mainly engaged in primary processing activities. They clean, gut, pack and freeze product, but very little is marketed as consumer-branded items and the volume of additional added value (e.g., recipe dishes, smoked products) is quite low. This is intentional. The market for highly processed trout in the U.S. is quite small. By keeping to volume production of their main lines, they achieve scale economies and keep costs down, retaining their strong national position. More specialist activities would in this sense be an expensive diversion.

*Specialist Salmon Trout Farmer Processors with Branded Products Aiming at Mass Markets.* These are companies who seek to derive their competitive strength from specialising in integrated production and thus, total quality control at all levels of their value chain. A successful Scottish example of this type is Strathaird who are integrated farmers, primary processors and smokers of salmon. They sell branded consumer and catering-pack smoked salmon products to consumer markets throughout the world. They lack a wide portfolio of different types of processed fish products but compensate with a strong specialist presence. Marine Harvest produces around 8,000 tonnes and has a 25% share of Scottish production. They are a subsidiary of Unilever, one of the major international food companies with expertise in the marketing and development of branded processed foods. This company has a diversified portfolio of activities from the sale of fresh fish to wholesale markets, to its activities in the retail sector and an extensive export trade. It has a processing plant for primary processing, which has now moved into higher value-added products. These developments are linked to its relationships with the multiple retail sector. As the volume of salmon available has grown, so there has been increasing emphasis on convenient forms of preparation for consumers. Marine Harvest prepare fillets, steaks and joints of salmon in controlled atmosphere packaging for sale under retail brand names and under their own brand name. They also market a range of smoked salmon products smoked for them on contract and then sold under the "Lochinvar" company brand name.

*Specialist Salmon Trout Farmer Processors with Branded Products Aiming at Narrow Markets.* Salmon and trout smoking are interesting examples of activities where firms of many different sizes are found. There are economies of scale in fish smoking and smokers supplying the major price-competitive markets tend to smoke many thousands of tonnes of salmon per annum. By contrast, the distinctive characteristics which can be created in the smoking process mean that smaller producers with specialist high quality products can often charge a premium to offset cost disadvantages. Regal Salmon of New Zealand (Fish Farmer 1992), currently producing 1000 to 2000 tonnes of chinook salmon, are remote from markets but are concentrating on supplying niche markets in the Far East, Japan and Europe for high quality "organically grown" chinook salmon that is filleted and cold smoked.

*General Seafood Processors with Farming Interests.* It can be an advantage to processors to offer customers, both consumer and wholesale, a portfolio of related products. At final consumer level, products with common company brand names can be promoted jointly, enhancing opportunities to create brand loyalty and reducing costs to achieve promotion impact. Thus, there are seafood companies such as BC Packers who have moved into salmon farming, either to secure supplies or because they saw it as an investment opportunity. Their strength is their wide range of branded fish products, of which salmon is one. This in turn has allowed them to build consumer loyalty and awareness in the North American markets. Recently Clear Springs Foods has been formed through the acquisition by Clear Springs Trout Company of the Coast Oyster Company for similar reasons (Seafood Business Leader 1992). Similar variety is found in trout processing. Sixty percent of output in Scotland goes to a co-operative, ScotTrout, which is owned by the growers and which guts and fillets fish for sale to processors or to retailers, but other major producers have concentrated on supplies to wholesale markets.

These examples demonstrate the central argument. There is no single definitive model for the organisation of salmonid farming and processing. The appropriate strategies depend on market opportunities, the nature of customers and competitors, and the management capabilities of the businesses involved. In making choices, therefore, it is necessary for the operation to have a very clear idea of why they are choosing a particular strategy, the competition they face, and the requirements they must meet to be successful.

## MARKETING: IMPLEMENTATION

### *Products, Production Planning and Pricing*

Products are central to the success of the business. They have many attributes including species, size, fat content, inputs, physical appearance, configuration, colour, packaging quality and methods, processing standards and methods, consistency across batches and over time, availability, volumes, and continuity.

The general issue of quality is an important one that merits detailed consideration. Quality issues embrace all aspects of growing and processing operations and thus represent a major management issue for the business as a whole. In competitive markets, without quality products the business is unlikely to survive and build customer loyalty. Affluent consumers are demanding consumers and standards of quality have been rising generally throughout the food business. Each business can be expected to set its own standards in this area.

This is also an issue which can be dealt with collectively. Fresh salmon and trout products are mainly sold unbranded and consumers cannot normally identify the individual producer. Where consumers are aware of origin, it is likely to be linked to particular countries or regions rather than individual growers. Good quality salmon will enhance the reputation of an industry while poor quality will have the reverse effect. In this situation all members of the industry have a common interest in ensuring that the overall reputation of the product is good. All the major salmon farming associations — the Norwegian Salmon Farmers Association, the Scottish Salmon Board, Irish Salmon Farmers Association, Shetland Salmon Farmers, B.C. Salmon Farmers Association and the Chilean Salmon Farmers Association, among others, have established quality control systems for their members. In Europe, individual country trout producers' associations belonging to the Federation Europeene de la Salmoniculture also operate quality control schemes and businesses of all sizes have participated in them. Their basis varies from voluntary schemes with limited arrangements for inspection, to ones with stringent quality specification and handling requirements and random checking through the distribution channel. These schemes include gill tagging fish to make them identifiable at all points in the marketing channel and the quality control scheme itself is used as a basis for generic promotion (*see* below). In addition schemes normally have some provision for the education of some members of the marketing channel who will be handling products. The stricter schemes are to be preferred because the output can be controlled, although in practice a number of trade associations have found it difficult to raise the finance and gain sufficient commitment from members to run such schemes. It is particularly difficult in times of low prices to gain co-operation from some producers who fail to see the necessity of such activities, often because of a lack of understanding of the contribution that it can make toward success.

Linked to the control of quality is a need to control the volume of production itself. Production and harvesting plans should be made on the basis of anticipated demand. This has two aspects, the first of which relates to short-term scheduling and production planning. It is one of the major competitive advantages of farmed salmon that such production control can be exercised, in contrast to the inflexibility of wild harvests. A major factor in the development of markets for farmed Atlantic salmon has been the ability to supply year-round. This lack of security of supply was a major factor inhibiting sales of farmed salmon in B.C. and has in the past led to the popularity of Atlantic salmon on B.C. farms, as this species can be better managed in this respect (BCSFA 1990).

Secondly, production planning has a long-term dimension. If proper market research is undertaken, it should be possible to estimate the volumes of production that should be available for each market, consistent with maintaining price levels. Where there is scope for further market development, production planning should be coordinated with plans for the development of that market. It should be emphasized that marketing is not an activity that takes place after production decisions have been made, but is an activity that should be part of the core decision-making processes in the business with production plans based on marketing plans and not the other way around. This issue is discussed further in the next section, which considers the problems resulting from a failure to plan properly in the 1980s and suggests some lessons for the future.

Pricing is a more difficult issue. For unbranded non-processed product, the individual producer is a price taker and does not have a great deal of discretion in determining prices. Suppliers with good reputations for quality and with established relationships with customers may achieve slight premiums. Clever bargaining can also bring rewards. On the whole however, the price is determined by market forces (i.e., levels of demand in relation to short-term market supplies). For branded products which are differentiated from competitors, the issue is more complex. In this area markets are highly segmented, as illustrated by the very considerable price range for smoked salmon products in Europe where retail prices can vary from US $25 to US $70 per kg. On the whole, if consumers consider a product to be of high quality or to have other additional characteristics which are valued, they will pay higher prices. Not all consumers value or are able to pay for these characteristics and, as a consequence, some seek different cheaper products. For the producer, therefore, the key issue returns to the choice of market segments which they consider themselves best suited to supply (i.e., the positioning of the product in relation to its competitors, and by remaining competitive within the chosen segment).

The only price regulation scheme which has operated in the industry was that of the now defunct Norwegian Fish Farmers Sales Association (FOS) for salmon and large trout. For many years FOS regulated minimum selling prices ex-farm. By Norwegian law all sales by farmers to exporters had to be invoiced through FOS, who could thus set minimum prices and check that these prices were not undercut. During periods of buoyant demand, this scheme probably led to more stable prices than would otherwise have existed (Shaw and Gabbott 1989), when in any case actual prices were way above minimum price levels. However, the scheme failed when the supply/demand balance changed. Surplus salmon put great downward pressure on minimum prices and despite reduction in official minimum prices, deals were made illegally outside the scheme at prices below the minimum level. The cause of failure, once again, was the lack of control over supplies. In spite of opposition from FOS, the Norwegian government had approved a large increase in licences to farm salmon in 1988, consequently increasing supply more rapidly than markets could be developed.

### *Promotion and Branding: Definitions and Roles*

Promotion includes advertising, sales promotion, publicity and personal selling. Its purposes are to inform customers, change attitudes or lead directly to additional or different purchases.

Individual businesses promote products to meet individual objectives of increasing their own sales or raising the prices at which they can sell. There are no statistics available to identify total expenditure by individual firms and, in any case, there are differences between businesses depending on the markets and customers selected. At the same time, industry discussions and monitoring of trade journals suggest a fairly common pattern which is reasonably consistent across countries.

For most salmonid businesses selling is the most important form of promotion. This does not only involve the process of negotiation. It requires the maintenance of contacts with customers, searching for new customers and monitoring the service given to customers. Effectiveness in retaining customer loyalty and sufficient knowledge of pricing levels to bargain effectively are of considerable importance to the overall profitability of the business.

Most advertising and public relations work by salmon and trout businesses is aimed at trade customers with whom the potential impact is greatest. Trade customers are more likely to have direct links with growers than are final consumers and are thus more likely to be responsive. At this level the number in the target audience is also lower than at the final consumer level, so that such exercises are less expensive than they would be at the consumer level. Media used are trade magazines, presence at trade fairs, and promotional literature, and the objectives are to inform and educate people as well as to remind them to buy the company's product. The extent of use varies and considerable ingenuity can be shown in finding ways of reaching customers in a cost-effective way. Farmers with small numbers of customers with whom they have frequent contact may consider that they have no need for such expenditure, while others with a wider customer network may find this an effective way of reminding existing customers and opening up contact with new customers.

For similar reasons, wholesalers tend to target their promotions towards trade customers rather than final consumers because of the lack of brand identities at the consumer level, but they do become involved in joint promotions to their customers together with their suppliers such as salmon and trout producers. Retailers and the food service sector are more likely to promote directly to final consumers, but the emphasis is likely to be on the business suppliers. There are exceptions to this. Where larger growers deal directly with supermarket groups, they can combine with the supermarket to promote the product to final consumers. This normally includes price promotions as well as advertising, demonstrations, and leaflets at point of sale. For fresh salmon, given the lack of company branding, the main impact is likely to be on the volume of salmon sold and the price level, but successful promotions also help to build loyalty of the retailer to the supplier.

The only possibility of creating consumer brand loyalty is when the origin of the product can easily be made known to the final consumer. There are some examples of where that has been achieved. Norsk Hydro through the MOWI brand have gill-tagged their products for many years and claim (Shaw and Gabbot 1990), although quantitative support is not available, that this has, together with some consumer promotion, enabled them to achieve a small price premium over competitors. A different example is Marine Harvest who have, as indicated above, developed a range of packaged and branded consumer products such as steaks, joints and fillets. In selected areas these have been advertised on television, in magazines and at point of sale.

More highly processed products with considerable differentiation such as smoked salmon offer the greatest scope for branding and the development of brand loyalty. As a consequence, these are more heavily promoted by individual companies, although they tend to make most use of point-of-sale displays, literature and magazine advertising, rather than mass media such as television on cost grounds (Table 13). It is here that the company with a range of products can make the greatest impact because the same brand name can be promoted simultaneously across products. Major French salmon smokers who smoke a range of different types of wild and farmed salmon carry out major promotions in supermarkets, emphasising range and overall quality of the company's products. As far as other media are concerned, the key to success is to select media which have a high percentage of potential consumers within their readers. Even in the largest European markets for smoked salmon, consumption is selective and mass consumer advertising is not cost effective.

TABLE 13

Promotional activity by major trout-producing countries (figures in US$ × 1,000 as of May, 1990) in the European Community.

| | Total revenue from sales (US$)[1] | Promotion budget (US$)[1] | Expense (% of total) |
|---|---|---|---|
| Belgium | 2,792 | 15.0 | 0.54 |
| Denmark | 87,459 | 10.8 | 0.01 |
| France | 93,080 | 1074.0 | 1.15 |
| Germany | 4,690 | 123.4 | 2.63 |
| Ireland | 4,316 | 5.8 | 0.13 |
| Italy | 87,558 | 463.0 | 0.53 |
| U.K. | 64,955 | 358.0 | 0.55 |

Source: Gabbott and Shaw (1991)

[1] in thousands

### *Branding and Generic Advertising*

This relatively limited capacity for individual promotion is not entirely satisfactory for salmonid producers. Because of strong competition from other food products, it is necessary to interest new consumers in salmon and trout in order to develop markets and to remind existing consumers in order to maintain levels of consumption in the face of so many alternative food choices.

It is this gap that can be filled by generic promotion. This is defined as a promotional campaign designed to benefit a product or grouping of similar products, without identifying brand names or origins of products at the individual business level (B.C. Salmon Farmers Association 1990). Generic promotion has similar objectives to those of individual business promotion in that it aims to stimulate demand by persuading non-buyers to buy, persuading existing buyers to buy more or by increasing buyers' perception of the value of the product. The difference is that the objective is now to stimulate demand for the product (salmon, trout) or the product of a particular group of producers, usually based on nationality (e.g., Irish Farmed Salmon, Danish trout). Generic promotion is widely used for many food products including salmon and trout. There are a number of reasons for this, including:

(1) It is difficult to build individual brand identities but it is easier to build product awareness; and

(2) By pooling resources across related producers it is possible to finance campaigns that individual businesses acting alone could not afford. The most notable examples are of the use of consumer advertising media, particularly television, although other activities such as promotion at trade fairs, and production of high quality brochures may also be easier to fund in this way.

Generic promotional work has been carried out by most of the trade associations in salmon farming. Some indications of the level of expenditure involved are presented in Table 13, which relates to the promotion of trout in Europe. Nevertheless, it should be noted that this is far less than the amount of money spent by major manufacturers of branded food products (Shaw 1990). For salmon, the largest spenders until their collapse in 1990 were the Norwegian Salmon Farmers Sales Organisation (Table 14) but other salmon organisations also spent substantial amounts, particularly in relation to their production levels. Expenditures rose substantially as production grew in the 1980s, partly because finance was more available and partly because of the need to develop new markets for rapidly growing production. Generally, such activity is considered to have been effective. The measurement of effects is complicated by the large number of variables involved, but the study by Bjorndal et al. (1991) confirms the positive response of demand in the French market to Norwegian generic promotion.

Generic promotion is difficult in the absence of a strong trade association. For this reason there has been little generic promotion of U.S. trout. Complications are also caused when products of different origins compete with each other in the same markets. Examples here are of competing promotions by Norwegian, Scottish, and Irish farmed salmon in the French market. As a consequence several attempts have taken place to pool activities and funds across producer associations to maximise impact on total demand for farmed salmon or trout. For instance in 1990, an initial attempt (B.C. Salmon Farmers Association 1990) to mount a combined advertising campaign in the U.S. market was not successful because of a lack of a commitment by a sufficient number of associations. However, the idea was succesfully reintroduced with the participation of the BCSFA and the Chilean Salmon Growers Association in a generic campaign in selected markets in the U.S. in 1994. Similar attempts have been made to promote salmon and trout jointly in the European Community by the Federation Europeene de la Salmoniculture, although they have also had difficulty in obtaining support from a sufficiently large number of associations. In principle such activities would seem a very effective way of stimulating overall demand. In practice, because trade associations have to account for use of funds to their members, it can be difficult to obtain funds for these activities. Members require direct impacts of expenditure. Often this can be difficult for the trade association because effects are often not immediate but rather represent an investment in building loyal consumers over time.

TABLE 14

Promotional expenditure (× 1,000) by Norwegian fish farmers' sales associations 1990.

| | Expenditures in Norwegian kroner | Expenditures in US$ |
|---|---|---|
| Country: | | |
| Japan | 248 | 40.9 |
| Italy | 560 | 92.4 |
| Sweden | 144 | 24.8 |
| Belgium | 190 | 31.4 |
| U.S. | 9,905 | 1,634.3 |
| France | 5,675 | 936.4 |
| Germany | 5,939 | 979.9 |
| Spain | 4,672 | 770.9 |
| Singapore | 3,411 | 562.8 |
| Activity: | | |
| Gill tag support | 5,410 | 892.7 |
| Other buyer support | 3,945 | 650.9 |
| Sponsorship | 3,880 | 6,402.0 |
| Merchandising trials | 891 | 147.0 |
| Exhibitions | 1,751 | 288.9 |
| Advertising and brochures | 4,424 | 730.0 |
| Agency services | 1,785 | 294.5 |
| Films and videos | 755 | 124.6 |
| Other | 1,480 | 244.2 |
| Administration | 4,334 | 715.1 |
| Total | 59,129 | 15,563.7 |

Source: FOS (1991)

## THE FUTURE: PROSPECTS AND STRATEGIES

### *Review of the 1980s*

Chapter 1 described the rapid increases that have taken place in production of salmonids, particularly salmon over the past decade. This development was an example of spectacular success in the development of new technologies for fish farming. There were linked successes in marketing with the development of major markets for the product. However, the success in marketing terms has been mixed, with a failure to analyze markets and plan development properly. This led to the crisis which has eliminated many farms from the business in the past few years, particularly in Europe, and threatened the future of large sections of the industry.

During the early part of the 1980s the biological and technical problems in farming Atlantic, coho and chinook salmon were largely overcome. Encouraged by high prices, spectacular growth in production occurred in the latter half of the 1980s. Atlantic salmon production rose from 48,000 tonnes in 1980 to 243,000 by 1990 while farmed Pacific salmon production rose from 4,000 tonnes to 54,000 tonnes. The consequence of this rapid increase was that the only way in which markets could absorb these additional supplies was through price reductions. The first reductions in prices took place in 1987 but since the growth of output slackened in that year, the first depression was short-lived and market prices had returned to former levels by the start of 1988 (Keogh 1990), encouraging further installation of new capacity. Fish farmers were encouraged by banks who were very willing to invest in salmon farms and by governments who offered various forms of financial support as well as technical service. The latter saw fish farming as a way of protecting threatened rural economies, particularly in Norway and in the European Community (Shaw and Bailly 1990). This created a situation of severe over-capacity in 1990. The problems for salmon farming were exacerbated by the success, after years of development, of the government-sponsored salmon ranching programs in Alaska and in enhancement programs in British Columbia, which led to large increases in wild Pacific salmon landings by the commercial fishery.

Oversupplies of salmon led to the failure of many businesses in Europe, particularly in Norway where the expansion of production has been greatest. They led to the collapse and bankruptcy of the Norwegian Fish Farmers Association which was the major controlling body for Norwegian sales and to reductions in the marketing activities of other trade associations, as members, fighting for survival, reduced funds for generic promotion activities. Further, they led to a growth in protectionism in world salmon trading, initiated by the levy of more than 24% on some exports of farmed salmon to the U.S. from Norway in 1990. As a consequence, most Norwegian supplies to one of its major markets were diverted to the European market, exacerbating the depression in European prices. The severity of the resulting crisis in the Scottish and Irish salmon farming industries, which are members of the European Community, have led to the introduction of minimum price levels for the sale of Norwegian salmon in European Community markets. While the depth of the crisis in other industries elsewhere has been less, most have experienced considerable problems because of the severely depressed prices.

Lately there have been "knock-on" effects on the markets for trout. While salmon and large rainbow trout have always competed relatively closely, the falling salmon prices brought salmon and portion-sized trout into closer competition with each other, depressing prices in the trout market, although in early 1992 there were signs of improvement (BTA 1991).

### *Prospects for the 1990s and Production Planning*

The future of the industry will depend to a considerable extent on whether it has learned from the harsh lessons of recent years. For salmon, the prospects for the development of markets are still good. In most countries, the percentage of consumers who eat farmed salmon is still relatively low yet it is a product with a good consumer image. Static or falling world landings of other fish mean that salmon is likely to be competing with generally rising world seafood prices, which should help to stimulate further demand. The markets for trout are more mature and probably the scope for substantial expansion are limited both by demand and by constraints on the supply side, although there are some prospects for further development.

However, to capitalise on this situation will mean that producers must take a market rather than a production-driven approach to their operations. We return to the elements discussed earlier in this chapter. The first is sensible production planning in the light of accurate demand analysis and with an objective of market development at sustainable prices, rather than the approach of first producing and then attempting to find markets. There are some signs of improvement. Forecasts for production (to 1995) suggest that in many cases we will see a reduction in production for the next few years and that production will only subsequently increase if market conditions permit. The experiences of earlier years have now led to banks being more cautious in funding businesses, particularly new ones. It is also likely that governments will be less willing to aid the development of new production for similar reasons. The European Commission, for instance, no longer aids new salmon businesses even in the most depressed areas of the Community. Depressed prices should have had the effect of shaking out many of the weaker producers. The stronger and more efficient businesses who remain should benefit from higher prices, which should ensure their survival.

In any commodity business matching supply to demand at prices that will ensure profitability for producers is not easy. Precise forecasting of demand is difficult, but supply decisions are further complicated by battles for market shares among competing producers. The two-year growing cycle as well as the time period to produce smolts or fry further complicates this situation. There remains a danger that future price increases, while unlikely to promote the levels of production increases of recent years, will lead to further cycles with periods of over-supply. There is no easy solution to this problem, although widespread availability to forecasts of both demand and growers' production intentions world-wide should provide some help. Recent experiences of the ease with which over-capacity can be created should also help to create more cautious attitudes.

There have been suggestions (Fish Farming International 1991) that there should be international agreements on capacity decisions. Whether such agreements would be acceptable to governments is another matter. On the whole the trend is away from regulation of markets, other than regulation through trade barriers. It is likely however that the resolution of this issue will lie with the individual business. The real test will be reactions when prices improve, and it is to be hoped that the need for caution in planning increased production and the need for proper market development will be remembered.

### *Businesses*

It was suggested in an earlier section that there were many ways of organising for successful marketing and for this reason, no one business structure is likely to emerge. There are likely to continue to be mixtures of vertically and non-vertically integrated businesses in the industry, precisely because market situations and channel structures vary between markets and because there is more than one efficient organisational solution.

Increased business expertise should make a contribution to the improvement of conditions. This in turn is closely linked to the question of types of organisation within the industry. It was suggested earlier that large businesses have certain advantages associated with factors such as quality of staff and access to market. In the more difficult trading conditions of the 1990s, access to these advantages will become even more important. Accordingly, further increases in concentration in business organisation can be expected, whether this is achieved through mergers or take-overs, or through the increased strengths of group marketing organisations. This is needed in order to improve the competitiveness of the individual business, contributing to the overall stability of the international industry. Larger businesses are more likely to recognise the international inter-dependencies of markets and growers within the farmed salmon business. They are also more likely to apply proper commercial criteria to their decisions, based on analysis of markets and competition.

A similar analysis can be made of trout production, with the exception of the local producers who supply local markets. Even in this case, however, such producers are likely to find themselves making more use of networks of relationships, formally and informally, with other producers. This is likely both for the supply of inputs and for the provision of common services ranging from joint marketing, where appropriate, to arrangements for joint supply of marketing services such as advice.

### *Activities*

The markets for fresh product sold in traditional ways are likely to remain important, as shown by the example of the more mature trout industry where traditional product forms still dominate. One part of the business will therefore remain closely linked to the traditional seafood distribution system. Since within that system there is relatively little product differentiation, remaining an efficient but high quality producer will be essential for success in this sector. The quality issue is particularly important and an area where improvements can be made. Even with the Norwegian system of quality control with mandatory inspection and detailed specifications, there are indications that poor quality and soft fish still find their way on to international markets (Seafood International 1991). However, high quality standards are the basis on which the other marketing activities of growers should be based and improvements need to be made to allow market development to continue. Given the role of price in determining demand, it is necessary to keep prices as low as possible with adequate profitability. Improved technologies and greater efficiency are likely to be very important if the basis of consumption in the population is to be widened.

It is likely that there will be some increase in the proportion of products which have value added in some form. In order to take advantages in trends in consumer demands particularly in Europe, there is likely to be an increase in the extent of product packaging and in the percentage of products that are made "convenient" for the consumer to use. There has already been major development in the range of value-added salmon products available and this is likely to be a continuing trend. In some cases these will be produced by the farmers themselves within integrated structures, but in other cases this will be done equally successfully by major food processing companies, not all of whom will be specialists in seafood. It is also suggested that there is considerable scope for the development of sales of processed farmed products, especially of cold-smoked salmon in other markets. These have small market shares elsewhere at present, but the experience in Europe gives some indication of the potential.

As markets for processed products develop, there are likely to be new ways of reaching the consumer. For instance, it will become much more common for salmon to be distributed and marketed within chilled food distribution systems handling a variety of foods of which fish is only one. Shorter distribution channels direct to retailers and the food service sector will become more common. With this development there are likely to be further changes in the way in which business is done. As growers develop better controls over their operations, the development of more efficient systems of product scheduling and ordering is possible and with it the development of closer relationships with customers, particularly but not only those in the multiple retail sector. This is likely to lead to more long-term approaches to price setting and greater stability in pricing, which will benefit profitability in the industry. It is also a way in which mutually satisfactory developments in pricing can take place. Egan and Kenney (1991) point to the tendency for distribution channels to absorb price reductions and note that the reductions that have occurred in ex-farm prices have not been matched by equivalent price reductions. They suggest that this is because of a failure by the retailer to understand that additional sales volumes at lower prices could be more profitable than smaller volumes at higher prices, given high consumer price responsiveness. Better communications and closer informal links in the distribution channel should help in this educational process. The emergence of larger businesses in the industry as suppliers in these channels will also provide countervailing bargaining power.

Packaged products are a way in which businesses can build distinctive images for themselves, but there is a general need to build awareness and interest in salmon and trout. This will be particularly important in those markets in which farmed salmon competes closely with wild salmon and therefore has to build a distinctive image as a high quality product. This is a major issue for the future in both fresh and smoked markets. The consumer is faced with an array of different types of salmon and is confused about differences, particularly in North America (Redmayne 1992). There are signs also that the marketing of wild Pacific salmons will improve. A positive response to these changes by producers of farmed salmon and trout is therefore necessary. There is a continuing need for promotion and education at the final consumer level. This is a need that has received relatively little attention so far. Most consumer advertising has been created by publicity about the industry and point-of-sale promotion. Even the Norwegians, with their large promotional budget, have concentrated mainly on trade promotion. As the industry becomes less newsworthy because it is no longer new, it will receive less free publicity. At the same time, if it is to extend markets by expanding the base of consumption in the population, it will be necessary to extend promotion and possibly make more use of visual media such as television.

Individual businesses will operate their own schemes which they will use to develop their own market positions. If they succeed in developing strong brand identities and consumer loyalty, they may individually feel that they have little to gain through collective promotion. In many ways this would be unfortunate because without collective effort it will be more difficult to use mass advertising media. Generic promotion of farmed salmon and trout has a continuing role in maintaining levels of awareness, and the scope for trade associations to continue their activities in this area is considerable.

A final aspect of this is the role of the industry's image in marketing. In recent years a growing problem has been the need for the industry to be a "good citizen" in terms of its relationship with the environment. This is necessary to stay within the regulatory framework. It is also necessary as part of marketing. It is difficult to promote a clean quality image for a product if the consumer does not perceive the industry to be operating in a clean environment whose quality it protects. The exact relationship between demand and concern for the environment is not completely understood and will in any case vary from country to country. However, the link would appear to be an obvious one.

## SUMMARY

This chapter has attempted to emphasize the necessary role that marketing plays in the profitable development of salmon and trout production. It has also sought to emphasize the central role to be played by marketing in business decisions. Major markets have been established for salmon and trout, particularly in the Northern Hemisphere, but opportunities exist to develop them further by extending development of new markets. As the industry seeks to take advantage of these opportunities there are likely to be many changes. To take advantage of these opportunities, stronger and larger businesses, and improvements in production planning and marketing, are required. At the same time, the differences between markets and the variety of different opportunities available to producers are likely to ensure that it remains an international industry of considerable diversity.

## ADDENDUM

World salmon production has increase considerably during the past three years, from 1.03 million tonnes in 1992 to an estimated 1.43 million tonnes in 1995. Farmed salmon's share of total salmon supplies has increased from 31 to 40% during this period. Norway has more than doubled its 1990 production of farmed Atlantic salmon to over a quarter of a million tonnes in 1995. Almost as significant in terms of production gains is farmed salmon and trout production from Chile, which will approach 100,000 tonnes in this year.

The majority of the increase Norwegian production has been exported to continental Europe, especially France, the largest salmon market in Europe. Increased Chilean production of farmed Atlantic salmon has been mostly exported to the United States, while farmed coho and trout production increases have gone mostly to Japan.

The increases in farmed salmon and trout production worldwide have come at a time of declining prices. Productivity gains in most regions have permitted production at lower costs. The farmed salmon and trout industries will continue to undergo cycles in markets, but these will become similar to the livestock industries, as opposed to the boom and bust cycle of the 1980s.

Several important food consumption and distribution trends continue to influence the marketing of farmed salmon and trout. Market conditions are generally favourable for farmed salmon and trout, however, a trend towards increasingly competitive protein markets is evident since the chapter was first prepared. The beef and pork industries, for example, have stepped up promotion campaigns in several western countries.

Consumers continue to demonstrate a willingness to pay significantly higher prices for meals which require less preparation time and as a result there is more evidence of value-added salmon production in all major markets. The salmon farming industry has also shown flexibility and creativity in adapting to environmental concerns of consumers. Single-generation sites and site fallowing is now extensively practised in Norway which has resulted in overall lower use of pharmaceuticals used in production.

Major structural changes are most apparent in the Japanese salmon and trout market. Salmon and trout distribution chains have been shortened considerably and Chilean farmed salmonid producers have made major inroads in this market, especially with portioned controlled cuts destined for the sashimi market.

U.S. salmon consumption is projected tom increase moderately. Most of this increased salmon consumption will be of farmed salmon. Price concerns remain the fundamental obstacle to increased purchases for home consumption, along with the fact that salmon appears to suffer from low "top of mind" awareness.

Indications are that generic promotion efforts with farmed salmon in the U.S. are likely to expand. Prices have tended downward and generic promotion remains one of the few significant factors driving demand that producers can directly influence. Over time, tariffs against Norwegian salmon imports in the U.S. are likely to be reduced, and competition in this market will intensify as a result. Future cooperation in generic promotion campaigns between the wild and farmed salmon industries is a possibility.

European salmon markets have continued to expand. In France, salmon is much more commonplace. Some limits to expansion of fresh salmon sales in the retail sector are being observed. There has been a significant increase in consumption of smoked salmon and salmon in other product forms, including canned salmon. Salmon is also being carried by a wider range of restaurants.

Oversupply situations from regions other than Norway now seem less likely to develop, in part because of more orderly market development. Some of the increases in supply have been the results of producers marketing on a year-round basis. In addition, market strategies being pursued by several companies are global in nature which has led to more concentration in selling.

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## Chapter 16

# ENVIRONMENTAL IMPACTS OF SALMONID CULTURE[1]

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## INTRODUCTION

### *Background*

Until very recently most research relating environmental quality and aquaculture was limited to assessment of environmental conditions necessary for culture. Emphasis was placed on dissolved oxygen requirements of the cultured fish or the maximum dissolved nitrogen levels that could be tolerated without impairment of growth rates or survival. Most attention was directed towards the effect of the environment upon the aquaculture operation, while the converse perspective, the effect of aquaculture upon environmental quality, was largely ignored.

The possibility that aquaculture itself could degrade water quality with adverse effects upon native flora and fauna received increasing attention in the 1970s with several investigations of environmental conditions downstream of land-based culture operations (Liao 1970; Hinshaw 1973). In a somewhat prophetic paper, Odum (1974) discussed several potential environmental impacts of aquaculture that had received little attention

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[1] This manuscript was submitted in March 1992 and the delay in publication was beyond the control of the authors. Readers wishing additional current information are referred to Hargrave (1994) for a discussion of modelling benthic impacts and Smith et al. (1994) or Weston (in press) for information on antibacterial usage by the aquaculture industry.

as of that time including nutrient enrichment, alteration of circulation patterns, and introduction of exotic species. With the rapid growth of salmonid cage culture over the past decade has come increased examination of this industry segment as a potential pollution source. In recent years numerous studies have appeared documenting environmental alteration in the areas surrounding salmonid cage farms. Most of the work has emphasized the benthic impacts of cage culture or, to a lesser degree, effects related to nutrient loading. Considerable information has now been amassed regarding the environmental effects of cage culture, but unfortunately many of the most comprehensive treatments appear in the "grey" literature or in publications with limited regional circulation (Weston 1986; Gowen et al. 1988; Håkanson et al. 1988; Rosenthal et al. 1988; Weston and Gowen 1988; NCC 1990; Mäkinen 1991*a*).

The potential environmental impact of aquaculture is not an issue merely of academic importance, but one with public policy ramifications. In Norway, the U.K., Canada, U.S., Chile, and, in fact, most areas where salmonid cage culture is practiced, the industry is now embroiled in considerable public controversy. Salmonid cage culture has provoked much of this debate because unlike the land-based facilities which have existed for decades, cages are often sited in public waters with a concomitant high public visibility and potential interference with other water uses. The phenomenal recent growth of this industry has often caught regulatory agencies unprepared with no specific permitting procedures, operational guidelines or monitoring requirements. The result has often been delay or denial of culture permits, enactment of moratoria to further industry growth, or exclusion of the industry from certain areas. Environmental issues have been central to this debate, and there is a clear need for objective scientific information on the environmental impacts of the aquaculture industry if growth is to continue in a sound manner.

### *Scope of Chapter*

This chapter reviews the available information on those environmental impacts of salmonid culture that have become the major elements of controversy in most regions and toward which most research has been directed. These issues include: (1) impacts on water availability (for those forms of culture where relevant); (2) impacts on the sediments and associated infaunal communities; (3) water quality impacts; (4) nutrient-related effects on phytoplankton or macrophytes; (5) effects on wild fish populations; and (6) the environmental effects of chemotherapeutants. Where appropriate, we have attempted to include information on pollutant fate (e.g., waste types and loading rates), environmental effects (e.g., changes in wild populations and communities), and mitigation methods (e.g., regulatory requirements, treatment technologies, predictive models). We have not included all environmental issues that have been raised, nor even all those that should be considered when assessing the potential impact of a given facility. For most other issues, however, data are nearly nonexistent or effects so dependent upon case-specific conditions that treatment here would be of little value. The environmental issues not included here are listed along with some of the best sources of information, when available: (1) alteration of water circulation patterns by cage structures; (2) introduction of exotic species (Rosenthal 1980); (3) effects on birds and mammals (NCC 1990).

The techniques used to culture salmonids throughout the world vary greatly particularly with respect to the water source (i.e., groundwaters or fresh, salt or brackish surface waters) and the means of confining the fish (i.e., raceways, tanks, ponds, cages). While addressing the environmental impacts of each culture technique individually would be impossible here, it is generally not necessary as, in many respects, environmental impacts are nearly independent of water source or confinement method. However, land-based culture vs. cage culture in open water is a major dichotomy central to the prediction of likely environmental impacts. A wide variety of waste recovery or treatment techniques are available to the land-based culturist where effluent is confined within some form of conduit. In cage culture the effluent is immediately diluted within the receiving water body with little or no opportunity for waste recovery and treatment. This difference is apparent in regulatory policies as well. A land-based salmonid farm is generally viewed by regulators as a typical point-source discharge. It will often be required to have some means of waste retention or treatment (e.g., settling pond or filtration), and the effluent will be regulated for parameters such as total suspended solids (TSS) and biochemical oxygen demand (BOD). However, a cage farm is subject to none of these treatment or effluent limitations, even if it contains as great or greater fish biomass as its land-based counterpart. Environmental impacts may therefore be different, and these two culture systems are treated separately throughout much of this chapter.

## TYPES OF WASTES ASSOCIATED WITH SALMONID CULTURE

### *Particulate Wastes*

The primary types of particulate waste from salmonid culture are feces and uningested feed pellets. When fed a dry pelleted diet, salmonid feces typically comprise about one-third of ingested material on a dry weight basis (Butz and Vens-Cappell 1982). The amount of uningested feed will depend upon many factors, including the feed type and method of dispersal, so consequently estimates of feed wastage vary greatly. Between 1 and 40% of the feed provided to the fish will not be ingested (Thorpe et al. 1990; Weston 1991). There is some evidence that feed waste is lower in land-based systems than in cages, possibly due to more efficient feeding in tanks or ponds (Beveridge 1987).

### *Nitrogen and Phosphorus*

Pelleted salmonid feed typically contains 1-1.5% phosphorus. The phosphorus in most feeds is both in excess of the dietary needs of the cultured fish and partially in an unassimilable form. Consequently, a substantial fraction of the phosphorus provided is lost to the environment via the feces, in addition to lesser amounts excreted in the urine.

Ammonia and, to a lesser extent, urea are the principal nitrogenous wastes associated with fish culture, and are produced as byproducts of protein metabolism. Ammonia may be present either as the non-toxic ammonium ion ($NH_4^+$) or as the toxic un-ionized form ($NH_3$). The relative proportions of the two forms are dependent upon temperature and pH, with formation of the toxic $NH_3$ favoured by high temperature and high pH. No cases of ammonia toxicity to aquatic life downstream from fish farms have been reported. In one survey (de L.G. Solbé 1982) the average $NH_3$ concentrations downstream of U.K. fish farms was 0.0016 mg/l, and more recent surveys in Scotland suggest that current levels are even lower (NCC 1990). These concentrations are far below the recommended 'safe concentration' of 0.25 mg/l (Alabaster and Lloyd 1982), suggesting that ammonia *per se* is unlikely to cause significant toxicity downstream of most freshwater salmonid farms. Similar conclusions have been reached by those studying marine cage culture (Weston 1986).

### *Dissolved Oxygen Depletion*

Salmonid culture will reduce dissolved oxygen concentrations through both fish respiration and mineralization of the organic-rich wastes (i.e., feed, feces, soluble metabolites). Salmonid respiration rate depends upon fish size, age, sex, activity and temperature, but an average respiration rate for routine metabolism is about 300 mg $O_2$/kg wet weight/h (Kils 1979; Liao and Mayo 1974). The BOD of the feces and metabolic wastes may consume about 1.5-3 times as much oxygen as respiration alone (Kalfus and Korzeniewski 1982; Liao and Mayo 1974; Willoughby et al. 1972). Effluent released from salmonid farms can deplete dissolved oxygen in receiving waters, either because the effluent itself is oxygen depleted, because of its high BOD, or a combination of both factors. There is also the possibility of indirect effects, such as nutrient-induced growth of micro- or macroalgae, and the eventual oxygen depletion accompanying decomposition of this algal biomass.

### *Chemotherapeutants*

Chemotherapeutants are employed to treat viral, bacterial, fungal, or parasitic infections of cultured salmonids. Parasiticides and fungicides are typically administered by an immersion treatment. The most commonly used parasiticide/fungicide in salmonid culture is formalin. It has been estimated that 60% of the freshwater salmonid farms in the U.K. use formalin (de L.G. Solbé 1982), and its use is widespread in other areas of the world as well. Malachite green is a highly effective fungicide also in wide use. de L.G. Solbé (1982) estimated it is used by about 40% of the freshwater salmonid farms in the U.K. In the U.S., however, concerns regarding its teratogenic properties and the persistence of residues in flesh and eggs have led to the prohibition of its use on food fish. Its use in that country is now limited to investigational use in federal hatcheries. Neguvon and Nuvan have been popular parasiticides used in marine cage culture, generally in the North Atlantic. Their use has resulted in considerable controversy regarding potential impacts on non-target marine species.

A wide variety of antibiotics are administered as feed supplements to treat bacterial diseases in salmonids. On a worldwide basis, oxolinic acid and oxytetracycline have historically comprised the vast majority of total antibiotic use by the salmonid culture industry, although their use has diminished in recent years. Other antibiotics used in one or more salmonid-producing countries include potentiated sulfonamides (Tribrissen®, Romet®30), flumequine, chloramine T and erythromycin.

Little is known about the environmental fate and effects of salmonid chemotherapeutants despite the fact that a substantial portion of the drugs often leave the culture site via the effluent, or in the case of cage culture, are directly released to the environment. Regulatory agencies have generally assumed that rapid dilution of the therapeutant would result in little or no environmental impact.

### *Microorganisms*

There have been few detailed studies of the bacteria present in the fish farm effluents. It is, however, known that the guts of fish can act as proliferation sites for Enterobacteriaceae, *Aeromonas*, coliforms and fecal streptococci that enter the culture systems through the influent water or feed (Trust and Money 1972; Trust and Sparrow 1974; Niemi and Taipalinen 1982; Austin and Allen-Austin 1985; Niemi 1985). Representatives of the gut bacterial flora are inevitably voided with the passage of food through the alimentary tract and are thus a component of fecal wastes.

## ENVIRONMENTAL IMPACTS OF LAND-BASED FACILITIES

### *General Description of Culture Systems*

Land-based salmonid culture systems in freshwater environments include hatcheries, systems for the production of fry and smolts, and systems for growth to consumption or restocking size. These systems may be integrated into one farm unit, or farms may specialize in producing one part of the salmonid life cycle (*see* Chapters 4 through 9 of this volume). The system or configuration of systems on the farm, in combination with site characteristics and management, play important roles in waste loadings and environmental impact.

Hatchery systems generally consist of baskets, trays or troughs in which the eggs are incubated. Following incubation, and after absorption of the yolk sac, salmonid young are weaned onto a pelleted diet, first in the hatching troughs and then usually in fibreglass tanks. Although there have been attempts to 'first feed' Atlantic salmon (*Salmo salar*) juveniles in cages in freshwater lakes (*see* Holm 1986 for review), most first feeding takes place in tank systems because of the need for close observation at this time, and an apparent behavioural requirement for moving water. Following this early stage, salmonids may be grown using a variety of land-based or cage systems. Land-based systems include tanks, earth ponds and raceways. Such systems typically are of the 'flow-through' type, but some 'recycle' systems are also in use. Recycling systems are used in fish farming when water availability is limited, or there is a need for strict control over the culture environment. The high cost of recycling systems has restricted their use in salmonid culture to a few hatcheries that heat water to accelerate egg development and then recycle the water to conserve heat.

Land-based systems for culturing salmonids in salt water are not common because of economic costs, but there are some tank and pond farms culturing Atlantic salmon and rarely, rainbow trout (*Oncorhynchus mykiss*) to market size in seawater (Bergheim et al. 1990). Information on waste loadings and environmental impacts of land-based, marine culture is scarce and therefore this review considers only land-based freshwater facilities.

### *Waste Products and Loadings*

Uneaten feed and excreta give rise to elevated concentrations of suspended solids, BOD, nutrients and minor elements in land-based salmonid farm effluent. There have been many studies of waste loadings from salmonid farms, some of which are summarized in Table 1. These studies show considerable variation in waste loadings, attributable to differences in species, fish size, physiological status, method and intensity of culture, and temperature.

Seasonal and diurnal fluctuations in waste output are common features of salmonid farm effluent. Waste loadings from hatcheries are likely to be small during egg incubation because there is no feeding. However, the use of formalin and malachite green to control fungal infection on eggs is common and, as a result, discharges of these substances may occur. After hatching, use of artificial feed results in increasing waste loadings from discharge of uneaten pellets, feces and soluble excreta. Because feeding rates are very high on a per unit biomass basis and feed is provided to excess at the first feeding stage in order to stimulate feeding, waste loadings per unit biomass are high. The very small feed and fecal particles produced at this stage can make traditional waste treatment difficult (e.g., settling ponds, screen filters).

Following early growth stages, salmonids will be transferred to different grow-out systems, the type of which affects total waste loadings. In general, feeding rates decrease

TABLE 1

Loadings of suspended solids (SS), biochemical oxygen demand (BOD), nitrogen and phosphorus from freshwater, land-based salmonid culture (from Beveridge et al. 1991).

| SS | BOD | $NH_4$-N | $NO_2$-N | $NO_3$-N | Total N | $PO_4$ | Total P | Ref. |
|---|---|---|---|---|---|---|---|---|
| (a) g·kg $fish^{-1}$·$day^{-1}$ | | | | | | | | |
| 0.0-7.1 | 1.6-2.7 | - | - | - | 0.1-3.8 | 0.02-0.27 | 0.01-0.43 | a |
| - | 1.4-8.1 | - | - | - | - | - | - | c |
| 0.8-0.9 | - | 0.3-0.8 | - | 0.13-0.21 | - | 0.07-0.17 | - | b |
| - | - | 0.3 | - | 0.05 | 0.12 | 0.033 | 0.10 | d |
| (b) kg ton·$fish^{-1}$·$yr^{-1}$ | | | | | | | | |
| 474-4015 | 510-990 | 37-180 | - | 0-548 | - | - | 22-110 | e |
| 1350 | 285 | 55.5 | 1.81 | 10.2 | - | - | 15.7 | f |
| - | 350 | 45 | - | - | 83 | - | 11 | g |
| (c) g·kg $feed^{-1}$·$day^{-1}$ | | | | | | | | |
| - | 80-300 | - | - | - | - | - | - | c |
| 183 | 165 | 25 | 0.27 | 0 | - | 26 | 4.0 | h |
| 80-280 | 100-370 | - | - | - | 37-48 | - | 4.7-10.8 | i |

[a] Bergheim et al. (1982); [b] Clark et al. (1985); [c] Butz and Vens-Cappell (1982); [d] Korzeniewski et al. (1982); [e] Alabaster (1982); [f] de L.G. Solbé (1982); [g] Warrer-Hansen (1982); [h] Butz (1988); [i] Mäkinen (1988).

but total feed inputs increase with biomass gains, resulting in greater waste loadings. During winter, when shorter day length and lower water temperature limits activity and feeding, wastage rates fall dramatically. A breakdown of the seasonal waste loadings associated with the annual production of 100,000 Atlantic salmon smolts [4 tons (t) of biomass] under typical Scottish conditions, indicates a considerable seasonal variation (Figure 1).

On a daily basis, waste loadings vary depending principally upon feeding schedules and tank, pond or raceway cleaning. Suspended solids, BOD and total phosphorus commonly peak during and immediately after feeding, later followed by peak ammonium concentrations. Several studies have shown that cleaning of tanks can result in 100-200 times the normal background solids load, due to sudden release of entrapped particles (Bergheim et al. 1982; Butz 1988; Beveridge et al. 1991). Ponds and tanks are known to act as sediment traps, with waste particles settling out, rather than being discharged in the effluent. A number of studies reviewed in Alabaster (1982) reveal a net reduction in solids concentration as water passed through the farm. However, accumulation of solids in ponds and tanks can lead to very high "shock" loads of solids during harvesting or tank cleaning (e.g., Boyd 1978; Ellis et al. 1978; Beveridge et al. 1991).

### *Environmental Impacts*

*Water Use.* Water requirements for land-based salmonid culture depend on stock biomass and feeding patterns. For a typical Scottish farm producing 100,000 salmon smolts annually, the periods of greatest water demand coincide with periods of lowest rainfall (Figure 2). Given the low flow rates of surface waters at this time, water quality impacts tend to be most acute during this period. In comparison with other food producing industries, water requirements for salmonid culture are high (Table 2).

Withdrawal of water for land-based salmonid farms has the potential to reduce water flow from streams and rivers (NCC 1990), with potential impacts including: (1) changes in channel shape, patterns of sedimentation, water movement and siltation; (2) loss of spawning areas for fish stocks, or loss of nursery areas; (3) barriers to the movement of migratory fish; (4) changes in biological communities, through loss of dilution capacity between inflow and outflow, reduced turbulence and oxygenation, plus possible loss of habitat due to stranding and desiccation in channel areas above the waterline.

The severity and extent of impacts depend on the proportion of total water flow withdrawn, as well as the distance between the farm intake and outfall. Problems are likely to be significant when low flow conditions combine with a high demand for water from the farm stock due to elevated temperatures (i.e., spring and summer in northern hemisphere). Problems associated with surface water withdrawal have been reported as acute in parts of southern England (Newbold et al. 1986; Jones 1990) where legislation

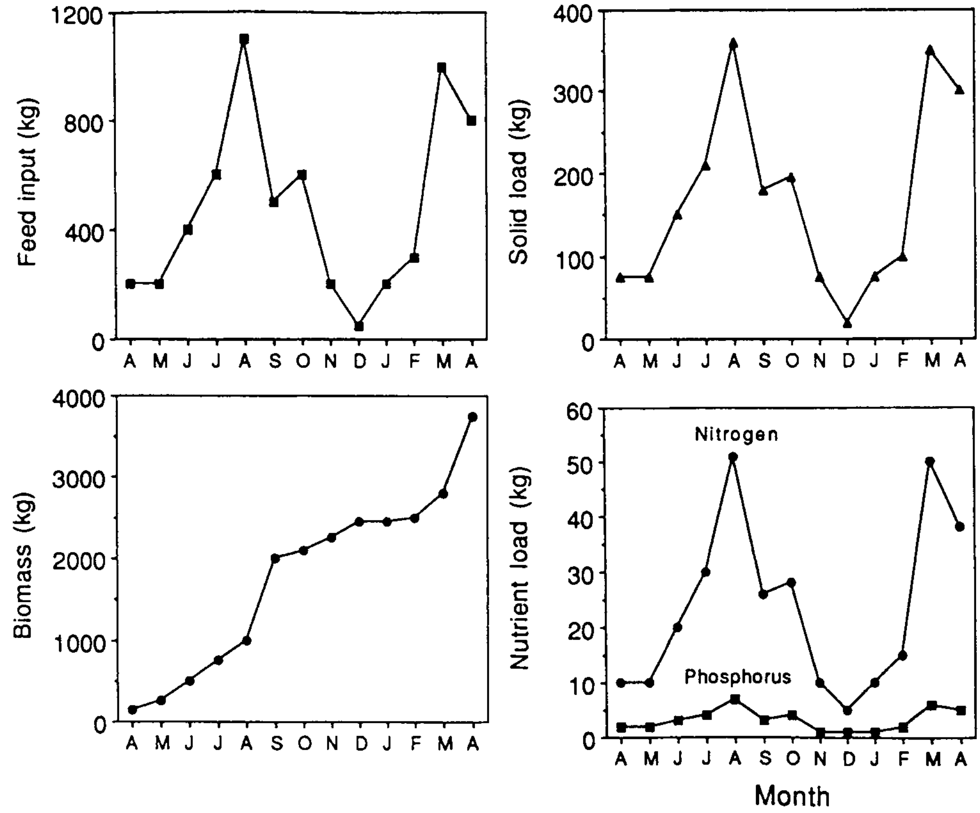

FIGURE 1. Total waste loadings from a typical Atlantic salmon smolt farm in Scotland. The figure shows the theoretical loadings derived from a production of 100,000 S1 smolts assuming a feed conversion ratio of 1.5:1 and 10% feed wastage, and assuming 50,000 potential S2 smolts are graded and removed in October of the first year (from Institute of Aquaculture, University of Stirling, unpubl. data).

does not control withdrawal rates in many circumstances. Some salmonid farms receive water from freshwater lakes (e.g., Orkney and Shetland Isles, Scotland; British Columbia, Canada), but no studies have been done on the impacts from this relatively uncommon practice.

Extensive use of groundwater for aquaculture has resulted in some groundwater salinization and subsidence in the U.S. and southeast Asia (Phillips et al. 1991). There is, however, no reported instance of such impacts specifically related to salmonid farming. Discharge of groundwater into running waters could enhance flow, which may be beneficial or harmful to native freshwater flora and fauna, depending on the relative amounts of water involved.

TABLE 2

Water requirements of salmonid aquaculture in comparison with other aquaculture systems, and industry and agriculture operations (adapted from Phillips et al. 1991).

| **Product** | **Water use ($m^3 \cdot t^{-1}$)** | **Product value ($US\$ \cdot t^{-1}$)** | **Water value ($US\$ \cdot m^{-3}$)** |
|---|---|---|---|
| Salmonids | 252,000 | 1,650-4,000 | 0.006-0.018 |
| Channel catfish | 6,470 | 1,650 | 0.25 |
| Shrimp ponds | 11,000-55,000 | 6,000-12,000 | 0.1-1.1 |
| *Clarias* ponds | 50-200 | 1,000 | 5-20 |
| Alcohol | 125-170($m^{-3}$) | 2,000($m^{-3}$) | 12-16 |
| Cotton | 90-450 | 1,000 | 2.2-11 |
| Beef | 42 | 2,000 | 48 |
| Pork | 54 | 2,000 | 37 |

*Dissolved Oxygen.* Various studies have shown that dissolved oxygen is depleted as water passes through a farm as a result of fish respiration and direct consumption by effluent wastes (e.g., de L.G. Solbé 1982). A survey of effluent from land-based tank and pond farms by Alabaster (1982) found a mean decrease of 1.6 mg/l from inflow to outflow, with an average flow of 12.6 $l \cdot s^{-1} \cdot t^{-1}$ of annual fish production. An identical oxygen depletion was recorded by de L.G. Solbé (1982) during a survey of trout farms in England. de L.G. Solbé (1982) also noted that only two out of 48 farms discharged water with an average dissolved oxygen concentration of less than 7 mg/l, indicating that most farms are unlikely to discharge water low in dissolved oxygen, as farms doing so would expose the stock to potentially stressful conditions.

Depending on the quality of the effluent, further changes in dissolved oxygen in receiving waters may occur. de L.G. Solbé (1982) found an average decrease in dissolved oxygen of 0.3 mg/l below U.K. farms, with less than 10% of the farms exceeding a 0.5-mg/l decrease. Sumari (1982) reported nine cases of downstream dissolved oxygen depletion in a survey of 200 fish farms in Finland, although it appears that this statistic refers mainly to lakes downstream of effluent outfalls. The need to maintain oxygen levels to protect the farm stock should ensure that significant depletion downstream from farms is unlikely in most cases, although the possibility exists of some localized depletion associated with deposition of organic solids.

*Chemotherapeutants.* Toxicity to downstream biota attributable to discharge of waste chemotherapeutants is possible, although there is little information on such effects. The few data available on the amounts of chemicals present in salmonid farm discharges suggest concentrations are low (de L.G. Solbé 1982; Beveridge et al. 1991). Formalin

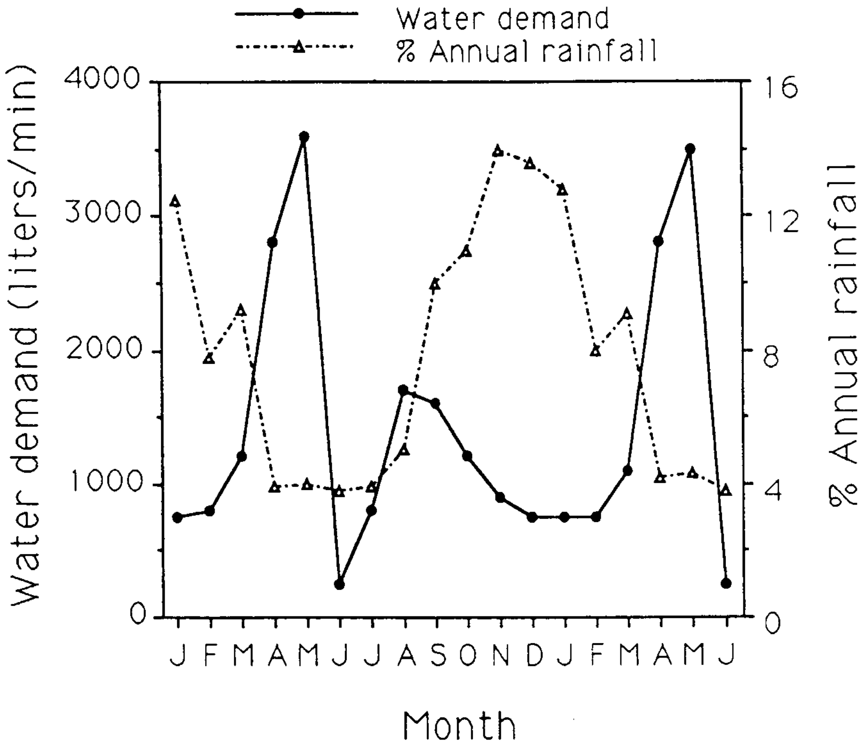

FIGURE 2. Seasonal rainfall patterns in relation to water demand for an Atlantic salmon farm producing 100,000 smolts per year under typical Scottish conditions (from Phillips et al. 1991).

(37% solution of formaldehyde) is widely used as an immersion treatment on tank, pond and cage farms for control of ectoparasites, usually as a bath treatment at 150-250 mg/l for 1 h. de L.G. Solbé (1982) estimated that an average U.K. farm used 12.4 l of formalin annually, and there is anecdotal evidence that some farms may use far more. Lethal concentrations of formalin vary from 60-600 mg/l for fish (for exposures of 24-96 h), 0.3-0.5 mg/l for algae, to up to 835 mg/l for certain aquatic insects (Bills et al. 1977*a*; Matheson 1981), suggesting the possibility of some localized toxic effects on aquatic biota directly below land-based outfalls, particularly for the more sensitive planktonic and microbial organisms.

Malachite green, another widely used chemical in freshwater salmonid farming, is used in the control of fungal infection and occasionally for the treatment of proliferative kidney disease (Clifton-Hadley and Alderman 1987). de L.G. Solbé (1982) estimated that on average 1.8 kg/yr was used per farm in the U.K. Malachite green is relatively unstable in the freshwater environment, where it is converted to the solid carbinol (Alderman 1988). This reaction will quickly lower concentrations of malachite green and reduce potential effects on freshwater biota. Short-term exposure to concentrations of 1-2 mg/l of active ingredient elicit a stress response in fish, although sublethal effects on fish respiration and blood chemistry have been reported at lower concentrations of 0.03-0.05 mg/l (Ross et al. 1985). Malachite green is a suspected teratogen at high concentrations. Meyer and Jorgenson (1983) found depressed growth, hatching delay and some skeletal deformities in rainbow trout eggs exposed to regular treatments of 1-2 mg/l. It is not clear if farm effluent concentrations of malachite green reach toxicologically significant levels.

*Microorganisms.* Some qualitative changes in the bacterial microflora of trout farm effluents have been observed, although the bacteria present are generally similar in terms of numbers and composition to those found in the inflows (Niemi and Taipalinen 1982; Austin 1985; Austin and Allen-Austin 1985). For example, Austin (1985) found that on only eight out of 29 sampling occasions were bacterial numbers in the effluent from trout farms substantially higher than those found in the inflows. Microbial diversity tends to increase with effluent nutrient content and decrease during and immediately after chemotherapy (Austin 1985). Although some studies have shown increases in the numbers of fecal coliforms during the passage of water through trout farms (Hinshaw 1973; Niemi and Taipalinen 1982), the data are fragmentary and the consensus seems to be that this phenomenon is not a significant problem. Detailed studies are still lacking, however, and the short-term effects of routine operations such as cleaning on bacterial numbers remains to be assessed properly. There is also no information on the presence of fish pathogens in farm effluents, and whether this release poses any threat to wild stocks.

Viruses have also been detected in farm effluents. Leong and Turner (1979) measured effluent concentrations of infectious hematopoietic necrosis virus (IHNV) as high as 400 plaque-forming units $(\mathrm{pfu})\cdot\mathrm{ml}^{-1}$ during a disease outbreak at a salmonid hatchery. While it has been shown that birds could act as mechanical vectors of pathogenic viruses from fish farms (Sonstegard and MacDermott 1972; Peters and Neukirch 1986), there is as yet little epidemiological evidence and their importance as vectors remains to be confirmed (Bregnaballe 1981; Beveridge 1989).

*Benthic Impacts.* Impacts of fish farm wastes appear similar to those associated with inputs of other organic matter and nutrients to freshwater rivers and streams (e.g., sewage - Hynes 1966). Effects include a loss of sensitive invertebrate species at or just below the point of discharge, with an increase in the density and biomass of organisms tolerant of organic pollution such as oligochaetes, chironomids and certain leeches. Several studies of salmonid farm effluent showing similar impacts on running water invertebrate communities have been documented (Munro et al. 1985). These studies show that effects are highly site-specific, depending on the type of farm, pattern and amount of background flow and volume and quality of effluent. Mantle (1982) and de L.G. Solbé (1987) both reported a loss of sensitive invertebrates directly below fish farm outflows in England. Markmann (1982) also reported a loss of "clean-water species" such as Plecoptera, Ephemeroptera and Trichoptera and an increase in oligochaetes, leeches, Diptera larvae and gastropods below Danish rainbow trout farms. Hinshaw (1973) investigated the benthic communities downstream from six hatchery outfalls and described a variety of responses, ranging from an increase in productivity to a loss of more sensitive species.

Organic-rich particulate wastes appear to be the most significant source of impact (de L.G. Solbé 1982), and there is evidence that benthic communities can return to background conditions if suspended solids are removed from effluent (NCC 1990). This recovery is conditional upon good maintenance of any effluent treatment plant, for poorly managed settling tanks have been found to exacerbate benthic impacts in Scotland as a result of enhanced release of dissolved BOD leading to a downstream proliferation of sewage fungus (M.J. Phillips, pers. comm.).

*Macrophytes.* Published data on the effects of land-based farms on macrophytes are limited, although enhanced macrophyte growth, particularly growth of pollution-tolerant species, is frequently cited as a response to fish farm discharges in English rivers (Allan 1983; Newbold et al. 1986). Sumari (1982) recorded increased macrophyte growth below three out of 200 fish farms. Mantle (1980) found little effect of a rainbow trout farm on the macrophytes of an English river, although the mosses *Brachythecium* sp. and *Leptodactum riparium* disappeared below the fish farm outflow. Studies on the River Hull show greater adventitious root growth and shoot extension in *Ranunculus penicillatus* var. *calcareus* collected below a trout farm discharge, although effects related to weed cutting may also have been important (Carr 1988; Carr and Goulder 1990).

*Wild Fish Populations.* Water withdrawal for land-based tank or pond farms may result in physical and chemical changes to fish habitats, and some loss of habitat has been reported in England (Allan 1983). There are also reports that descending Atlantic salmon smolts may be caught in fish farm inflows (Allan 1983). The scale of such impacts will depend on the volume of water withdrawn, the distance over which the river flows are reduced, inflow design, and the cross-sectional area of the river. Alabaster (1982) found that downstream fisheries were not affected by fish farm effluent provided the total flow of the river receiving the effluent was above 5 $l \cdot s^{-1} \cdot t^{-1}$ of fish production. de L.G. Solbé (1982) also found that deterioration of fisheries downstream from land-based fish farms (mostly in England) was rare. Studies in Denmark (Rasmussen 1988) and the U.S. (Hinshaw 1973) showed that addition of fish farm effluent may increase the productivity of downstream fish populations.

### *Waste Reduction and Treatment*

*Feeding Techniques and Feed Type.* Careful feeding and the use of correct diet offer good potential to reduce effluent loads at the source. Although feed losses have been reduced significantly in recent years due to economic pressures (de L.G. Solbé 1987), such losses may still form a substantial part of the overall waste load from a fish farm. Overfeeding of fish also decreases feed digestibility and increases fecal production. Thus, a reduction in feed losses by monitoring of feed losses and careful hand-feeding, either exclusively or as a supplement to automatic feeders, can significantly reduce effluent loads and reduce impacts on running waters (Bromage et al. 1990).

The physical characteristics of the fish food are very important in terms of pollution potential of the feed. The use of dry pellets rather than moist pellets or "trash" or "rough" fish considerably reduces the amount of wastage (Alabaster 1982). Hardness, disintegration rate and colour of the pellets are also important, as they affect palatability and the stability of fish food in the water. Poor palatability will result in more rejected pellets and a higher load of uneaten food to the environment. Unstable pellets may also increase waste loadings if rapidly broken down into unacceptably small-sized particles. Lack of symmetry in the food pellets is to be avoided as much as possible to allow uniform settlement in settling basins (Price and Clements 1974). Food with low settling velocity also helps to prevent excess wastage (Agiovlassitis 1988).

Manipulation of diet composition has been demonstrated to be an efficient and cost-effective means of reducing ammonia and soluble BOD output (Henderson 1988), and there is considerable scope for reducing phosphorus loadings (Matty 1990). Fish fed on so-called 'low pollution' diets have also shown better food conversion ratios and growth rates as well as improved resistance to disease (Phillips et al. 1988; Roberts 1989). Different constituents of artificial diets are linked to effluent quality (Table 3).

The amount of phosphorus discharged from fish farms is determined by the amount and digestibility of phosphorus in the feed (Ketola 1982; Crampton 1987). The total concentration of phosphorus must be kept low and its digestibility high to minimize waste phosphorus release. Most waste phosphorus is bound in the particulate fraction, although a significant part of this particulate fraction is easily dissolved. Dissolution is affected by several factors, including solid surface area, degree of agitation, concentration gradients, temperature and other water quality factors, as well as the composition of the feed. Mobility of phosphorus in fish food and feces can differ greatly between fish feeds (Carlsson 1988).

Recent studies by Wiesmann et al. (1988) have demonstrated that food conversion rates were unimpaired if the phosphorus content was reduced from 10 to 4 g $P{\cdot}kg^{-1}$ dry feed and Finnish work has also shown 4-5 g P $kg^{-1}$ to be an adequate concentration for rainbow trout (Matty 1990). Limited studies with Atlantic salmon suggest a dietary requirement of 3 g $P{\cdot}kg^{-1}$. Recent reports suggest phosphorus levels in salmonid diets

TABLE 3

Dietary characteristics contributing to increased waste loading in fish farm effluent (Henderson and Bromage 1987*b*).

| **Waste type** | **Dietary characteristics** |
|---|---|
| Suspended solids | indigestible solids<br>excessive insoluble carbohydrates<br>poor quality carbohydrate sources |
| Biochemical oxygen demand | indigestible biodegradable solids<br>excessive carbohydrates<br>poor quality carbohydrate sources<br>excessive oil<br>poor quality oil |
| Ammonia | excessive protein<br>poor quality protein sources<br>insufficient fat<br>insufficient carbohydrate |
| Visible oil | excessive oil<br>poor quality oil |

are 12-16 g $P\cdot kg^{-1}$ (NCC 1990). There would thus seem to be excellent potential for reducing phosphorus levels in salmonid farm effluent by reducing phosphorus in feed.

Nitrogen excretion depends on many factors including its bioavailability and feeding rate, but on average, 60% of dietary nitrogen is excreted (Beamish and Thomas 1984). The quality of fish meal and other protein sources used in the diet dictates the proportion of feed protein that can be assimilated into muscle tissue. Ideally, protein is used to build muscle, while energy requirements are satisfied with highly digestible and available fat and carbohydrate sources, rather than with protein. Ammonia excretion rates are higher if protein is used as an energy source, because ammonia is a by-product of protein metabolism. The proportion of ammonia excreted relative to that of ingested nitrogen increases with dietary protein concentration (Beamish and Thomas 1984), while excretion of urea nitrogen is less dependent on dietary protein or lipid concentration.

Poor quality carbohydrate sources result in increased suspended solids and BOD waste. When in excess in the effluent, carbohydrates increase the BOD and can cause growth of sewage fungus in receiving waters. Alternatively, if the carbohydrate (or lipid) source is insufficient, then ammonia and other nitrogenous wastes increase. In the production of extruded pellets, the higher temperatures and pressures may result in gelatinization of dietary starch, thus increasing the bioavailability of carbohydrate. This alteration is therefore advantageous providing the available carbohydrate level does not exceed the maximum tolerable level and impair liver function (Hilton et al. 1981).

Oils and fats may contribute to visible surface scums and the BOD of fish farm effluent (Table 3). Problems associated with these constituents can be partly avoided by the use of high quality ingredients and a correct balance between requirements and concentration in the diet. Many freshwater diets are formulated as "high energy" diets that contain high levels of fat. These diets are designed to minimize protein metabolism and can be used to reduce ammonia excretion.

*Settlement Treatment.* Treatment of fish farm effluent to reduce solids and nutrient loading and to correct dissolved gas levels in order to reduce environmental impacts has become increasingly important. In some areas of the world, regulatory authorities are insisting on waste treatment (van Houte et al. 1989; NCC 1990); in others, farmers have a statutory limit on effluent quality, and increased fish production is possible only by introducing some form of treatment. There is insufficient space in this chapter to deal with all the issues involved in waste treatment in aquaculture and interested readers are recommended to consult Wheaton (1977), Muir (1982) and Hugenin and Colt (1989). The present account is primarily concerned with the more commonly used systems for salmonid hatcheries and production units.

Settlement treatment systems work on the principle that solid particles with a density greater than water will fall out of suspension when water flow is reduced. The rate at which particles will settle in still water conditions depends largely on particle size and density; larger or more dense particles will settle more rapidly than smaller or less dense ones. The design and effectiveness of a settlement system is therefore dependent on the retention time of effluent in a settling tank or pond as well as the surface area available for settling (Wheaton 1977; Bromage et al. 1989). It is also desirable to prevent the solids in the effluent becoming fragmented as particle break up will inhibit settling and promote leaching of nutrients from the solid waste. Fish farms and settling tanks should be designed to minimize break up due to unnecessary turbulence.

Early studies (Liao 1970; Liao and Mayo 1974) indicated that "lagooning" (simple settlement ponds) was a reasonably efficient method for reducing the BOD in freshwater fish farm effluent. The results from more recent studies show that up to 90% of suspended solids, 60% of BOD and 50% of total phosphorus loads can be removed by settlement treatment, although system performance is extremely variable (Table 4). One major problem with settlement tanks is that removal of suspended solids from fish farm effluent is related to the concentration in the influent to the settling basin. When levels are <10 mg/l, as is common in salmonid farm effluent, efficiency is greatly reduced, although it is possible for suspended solids to be increased by pre-concentration treatment (Henderson and Bromage 1987*a*). It is also difficult to obtain suspended solids

TABLE 4

Percentage removal efficiency figures for settlement treatment (review of available data; from NCC 1990).

| Suspended solids | BOD | Total phosphorus | Retention time (min) | Reference |
|---|---|---|---|---|
| 69 | | 47 | | Dove-Edwin (1989) |
| 36 | | | 4 | Alabaster (1982) |
| 16 | | | 7 | " |
| 56 | | | 80 | " |
| | 8.2 | | 360 | " |
| | 60-80 | | 240-600 | Liao (1970) |
| >60 | | | >3 days | " |

levels of <6 mg/l in settled effluent (Henderson and Bromage 1988). When suspended solids levels are low, resuspension of particles within the settlement pond accounts for a large proportion of the solids present in the outflow. Other problems are that the area required for settling ponds or lagoons can be large in comparison with the size of the farm.

Another class of settling tank design is based on a circular water flow (centrifuge) to increase the gravitational force experienced by particles during settlement (*see* Wheaton 1977 for discussion). In a conical, scroll-type, continuous centrifuge, the water to be treated enters through the centre of the unit. The liquid and suspended solids are thrown out to the centrifuge wall, where a conical screw auger removes the solids from the centrifuge.

The swirl concentrator is another type of settlement device that has attracted some interest, primarily in continental Europe. A swirl concentrator is a circular tank in which the flow of water is directed tangentially so that solids are drawn towards the bottom of the tank and are removed, while the treated water is separated via an overflow weir (Warrer-Hansen 1982). This technique has been advocated in Denmark for effluent treatment from land-based rainbow trout farms, although very few are actually in use. In de L.G. Solbé's study (1982) less than 2% of farms studied in the U.K. used such a device. One of the advantages of this type of treatment is its compactness, although this advantage is often lost because it is commonly used in conjunction with settling ponds. Evidence as to the efficiency of this device is contradictory. Leffertstra and Kryvi (1988) found the swirl concentrator was only moderately effective, its advantages being its low cost when considering hydraulic capacity and its lack of moving parts. It is very dependent on gentle water flow, has problems with clogging and it is sometimes difficult to maintain the correct vortex necessary for maximum waste removal. Due to algal growth it also requires cleaning 1-2 times per week (Leffertstra and Kryvi 1988). It is unlikely that this system will see widespread future use. Its efficiency is not as good as that of the "Triangelfilter" (Mäkinen et al. 1988) and a well-designed settling pond eliminates any requirement for a swirl concentrator (Henderson 1988).

A major constraint upon the use of settlement devices remains the characterization of particle size of waste loads; as previously mentioned, both the nature and quantity of wastes produced by a farm varies substantially both during a day, and throughout the growing season. A consequence of this varying waste output is that in order for settlement devices to be effective waste treatment systems, they must be designed to operate efficiently over a wide range of particle sizes.

*Screening and Filtration Treatment.* Salmonid farm effluent may be treated by passage through a screen to remove particulate matter. A single stationary screen may be suitable for treating effluent which does not have continuously high solids levels (Mantle 1980). A more effective alternative is to use several screens of decreasing mesh size (e.g., 2, 1 and 0.5 mm). These screens should be easily removable to allow for cleaning, repair, or substitution by other mesh sizes. All static screens require regular cleaning, and where large quantities of solids are present in the water, labour costs may become prohibitive and severe problems may be encountered with blockages and reduced water flows. Such problems often arise with fish farm effluent.

The alternative to a single stationary screen is a self-cleaning or rotating filter. The most common configurations are variations of rotary screens, where the screen operates only partially submerged in the water that is to be filtered. The submerged section of the screen filters the water passing through it while the remainder of the screen is cleaned, usually by a high pressure water jet, with the residue running to a settling pond. The clean section of screen then rotates to replace the submerged section. There are two basic types of rotary screen: axial flow and radial flow. In an axial flow screen, the water passes through the screen parallel to the axis of rotation. In a radial flow screen, the water flow is towards or away from the axis of rotation. Examples of these screen types together with some variants are described in Wheaton (1977).

Removal efficiencies for one of these systems, the "Triangelfilter" (trade name), are presented in Table 5. These data clearly demonstrate the potential of these and similar screen filters for removing materials from fish farm effluent.

After screening, filtration may be used as a secondary system for fine solids removal. Diatomaceous earth filters are good at removing extremely fine particulate matter (0.1-5 $\mu$m), but are not cost effective in treating effluent from salmonid farms. The most common filter medium is sand and gravel ranging in size from 0.25 to 5 mm, usually graded coarse to fine in the direction of water flow. Slow sand filters use ungraded sand media and have a low filtration rate. They have the advantages, however,

**TABLE 5**

"Triangelfilter" waste removal efficiencies (percentage).

| SS | TP | BOD | TN | COD | DOC | DRP | Reference |
|---|---|---|---|---|---|---|---|
| 90 | 80 | 70 | 40 | | | | manufacturer |
| 80-87 | 67-79 | 75-82 | 27-40 | | | | Enquist and Larsson (1985) |
| 80 | | 85 | | 75 | | | Heerfordt (1987) |
| 76 | | 53 | | | 26 | 55 | Kadri (1988) |
| 55.5 | 18-66 | 32.3 | | | | | Dove-Edwin (1989) |
| 40-94 | 41-83 | | | | | | Goldberg et al. (1987)[1] |

SS=suspended solids, TP=total phosphorus, BOD=biochemical oxygen demand, TN=total nitrogen, COD=chemical oxygen demand, DOC=dissolved organic carbon, DRP=dissolved reactive phosphorus.

[1] Different models and screen sizes used by these authors.

of having a modest head loss and of combining some biological filtration with mechanical filtration. Filtering efficiency, in general, increases as media size decreases.

Settlement and filtration systems each have advantages and disadvantages. The advantage of the "Triangelfilter" or similar systems is that solids are separated from the effluent water relatively quickly, thereby reducing the amount of time for leaching of soluble material from solid particles. With settlement treatment, the solids remain in contact with the water after settlement, and it is possible for the concentration of dissolved waste products (particularly ammonia and dissolved reactive phosphorus) to increase as the effluent passes through a settling tank (Dove-Edwin 1989). This increase may not be a problem where reduction of solids levels is the primary consideration.

The growing concern over potential impacts of therapeutants on the environment has stimulated interest in techniques for removing such chemicals from fish farm effluent. Experiments on the removal of malachite green from hatchery water have shown that filtration through some form of activated carbon (charcoal, peat) may be effective (Bills et al. 1977*b*; L.F. Marking, pers. comm.). There is little information on methods for treatment of other chemicals and further investigation in this area is required.

*Biofiltration.* Biofiltration can, in theory, be used to improve effluent water quality from salmonid farms. In aquaculture, biofilters are commonly used in recycle systems to prevent accumulation of ammonia and nitrite (Mayo 1991). The technique is most appropriate where concentrations of pollutants are high or effluent volume is low (de

L.G. Solbé 1987) and is not considered practical or economic for treatment of salmonid farm effluent in most circumstances due to low temperatures (NCC 1990) and the large volumes of effluent involved (Henderson and Bromage 1987*a*).

There has been some interest in using algae and aquatic macrophytes, such as reeds, to reduce levels of nutrients in effluent. Reed beds are being investigated for nutrient removal from small scale sewage works (Cooper and Hobson 1989) and water hyacinths and duckweed have been grown for this purpose in warmer countries (Zirschky and Reed 1988). The application of such techniques requires more detailed consideration. For example, die-off of plants during winter could result in stored suspended solids, BOD and waste nutrients being released unless plants were harvested before winter. Slow growth in some climatic conditions could also inhibit nutrient uptake (Henderson 1988).

Most recently, biofilters that rely upon the development of a bacterial population on a large surface area medium (usually some form of plastic substrate) have been used both in the U.K. and Denmark with varying degrees of success. The technique relies primarily on the oxidation of ammonia to nitrite and nitrate. The success of such systems in reducing outputs of other than nitrogenous aquaculture wastes is not well documented. In all cases, the biofilter is used "in line", as either a front or back end to other conventional systems (e.g., settlement ponds, rotating filters).

As with settlement ponds, one of the major constraints to biofilters is that of space required. Some biofilter designs require large areas of land to be set aside for this purpose and such installations may be outside the economic capabilities of many farms. In all cases, the performance of biofilters is limited by operational constraints within the farm itself; use of therapeutants or sudden changes in water flow may impair or halt operation of the filter altogether. Use of biofilters undoubtedly requires a good degree of knowledge regarding the functioning of the system by the farm operators themselves in order to ensure continuity of operation.

*Other Treatment Systems.* Post-farm aeration is commonly used in larger trout farms throughout England and Denmark as a means of ensuring compliance with regulatory requirements pertaining to dissolved oxygen concentrations in the effluent. Impeller-type aerators are generally used, and operation is controlled remotely by an oxygen sensor, which activates the aerator once outflow oxygen levels are detected below a fixed level. While there is interest in using other systems to treat salmonid farm effluent, such as foam fractionation, ozonation, chlorination and dechlorination, and ultraviolet light, there is little information on the practical effectiveness of these methods for conditioning effluent, and many of these treatments are used primarily as measures to ensure good inflow water quality.

*Waste Utilization.* Most common forms of solid treatment generate a sludge requiring periodic disposal. In Scotland, the sludge from settling ponds or tanks is usually removed once per year (at a cost of about US$ 400) or when there is evidence that accumulation of sludge is adversely affecting the quality of water passing through the pond (NCC 1990). Some difficulties may be encountered due to the high water content of the sludge, but after drying it can be utilized as a fertilizer on agricultural land (Mäkinen et al. 1988) or forestry land (de L.G. Solbé 1988). The sludge can also have a moderate neutralizing capacity in a strongly acidic soil. Willett and Jacobsen (1986) found trout farm waste in Australia to be a source of available nitrogen and phosphorus to crop plants. Problems have arisen in Scotland with this type of sludge disposal, however, including organizational problems of having the sludge ready when the demand arises, and claims that the sludge induces more weed growth than would be expected with conventional fertilizers. There has, in addition, been some resistance to this usage because of odour problems.

### *Impact Mitigation*

*Government Regulations.* Regulations aimed at controlling the environmental impact of farm effluent and preventing excessive withdrawal of surface and groundwaters exist in several countries (van Houte et al. 1989; NCC 1990; Phillips et al. 1991). In some countries (e.g., U.K.), permissible effluent concentrations are set depending on the quality of the water receiving the effluent, and standards may be more stringent for rivers and streams of higher water quality (NCC 1990). As with many other countries in the European Economic Community (EEC), land-based farms in the U.K. are regulated primarily on the basis of ammonia, suspended solids, BOD and oxygen concentration in the effluent water (Table 6). Permissible concentrations of effluent wastes are often specified as an absolute maximum rather than on a net change between inflow and outflow. In Scotland, as distinct from England and Wales, farms are limited on the basis of the quantity of discharge water they may release into a channel, whereas in England and Wales, regulations exist to cover licensing of both intake and discharge volume from a fish farm. Licensing authorities may also include other conditions regarding quality of effluent covering a variety of compounds (e.g., malachite green, formaldehyde, antibiotics). All such additional conditions are discretionary and normally set with regard to the perceptions of the licensing authority, and within the limits of their ability to analyze and monitor for such compounds. These discretionary powers extend to specifications for outfall positioning and the installation of treatment devices. In many European countries, licenses specify the installation of a settlement device in the farm outfall which has a >25-30-min residence time (Böhl 1991; Heerfordt 1991).

Once standards are established, monitoring of effluent may take place to ensure that parameters remain within permissible concentrations. However, problems exist in monitoring salmonid farm effluent because of the considerable diurnal and seasonal variations in waste loading (e.g., loads during tank cleaning or feeding may be significantly higher than normal background loads). Presently in the U.K., effluent compliance sampling is undertaken on the basis of single "spot" samples and can, as a

**TABLE 6**

Parameters regulated in land-based salmonid farm effluent (with special reference to the U.K. regulations).

| Parameters | Typical permissible levels (mg/l) |
|---|---|
| Total ammoniacal nitrogen | $< 0.4$-$0.5$ |
| Biochemical oxygen demand (BOD5) | $< 1$-$3$ |
| Suspended solids | $< 5$-$10$ |
| Dissolved oxygen | $> 5$-$7$ (80% saturation) |

result, seriously under- or overestimate the typical effluent quality of a farm. Such problems have been minimized in some countries (i.e., Denmark and Austria) by the use of continuous, automated water samplers (Butz 1991; Heerfordt 1991). In remote sites, where such a level of surveillance is not logistically possible, increased reliance on macroinvertebrate biomonitoring may be considered. Denmark is presently one of the few countries in Europe specifying feed quality and quantity as part of the standard farm licensing procedure.

*Predictive Models.* Models for assessing water flow and quality have potential as tools for assessing the suitability of a site for fish farm development and for predicting impacts. For example, in Scotland, water availability is calculated on the basis of the farm withdrawing 30-50% of the dry weather flow (NCC 1990). Low-flow predictions are made to ensure sufficient supply for wild fish stocks, dilution of effluent, and to avoid stranding and desiccation of running water habitats. The most commonly used low-flow calculation for water users is the Q95(10), the 10-d average flow exceeded by 95% of 10-d average discharges, which is calculated from known catchment characteristics and 25-yr rainfall records (Institute of Hydrology 1986).

The effects of land-based salmonid farms on river water quality are commonly judged on the basis of BOD, ammoniacal nitrogen and suspended solids, although phosphorus may also be used in oligotrophic waters (NCC 1990). Downstream water quality can be assessed using: (1) fish farm waste output (mass flow); (2) river flow; and (3) upstream water quality. The three factors can be put together in the following equation (using BOD as an example):

$$\mathrm{BOD(d)} = [(\mathrm{Q(u)} \times \mathrm{BOD(u)}) + (\mathrm{Q(e)} \times \mathrm{BOD(e)})] / (\mathrm{Q(d)}) \qquad (1)$$

where BOD(d) = BOD concentration downstream (mg/l); BOD(e) = BOD concentration effluent (mg/l); BOD(u) = BOD concentration upstream (mg/l); Q(u) =

upstream discharge ($m^3 \cdot s^{-1}$); Q(d) = downstream discharge ($m^3 \cdot s^{-1}$); Q(e) = effluent discharge ($m^3 \cdot s^{-1}$).

The predicted BOD (or other) concentration in the effluent can be obtained from figures available on effluent loading (*see* page 1016). The predicted concentrations downstream for particular parameters can be compared with water quality criteria to assess impacts (Carr 1989). There are several problems with this approach: the actual amounts and types of waste produced by fish farms are extremely variable depending on the type of system and management, and there are seasonal and daily variations in load. Even so, such an approach has proved useful in predicting broad-scale water quality changes and in indicating where potential problems may exist.

Alabaster (1982) has indicated that adverse effects on fisheries and localized impacts on flora and fauna may exist if the water flow in the receiving water is less than $5.0\ l \cdot sec^{-1} \cdot t^{-1}$ of annual fish production, with slight or no impacts likely above $13.4\ l \cdot sec^{-1} \cdot t^{-1}$ of production (Table 7). Such basic calculations can prove useful in quickly assessing potential impacts.

## ENVIRONMENTAL IMPACTS OF FLOATING CAGE CULTURE

### *General Description of Culture Practices*

Cage culture, also known as net-pen culture, consists of a net suspended in the water column with a flotation system around its perimeter. Most often the net is hung in a square or rectangular configuration (four sides and a bottom), but some cage systems employ circular nets. The size of a cage can vary considerably depending on the needs of the culturist: a small cage may enclose an area of only a few square metres; larger cages, particularly those intended for use in offshore areas, may enclose 500 $m^2$. The depth of the cage usually varies from 3 to 15 m. The cage is moored by one or more anchor lines extending out from the perimeter.

In most cases a farm is comprised of multiple cages, either moored in close proximity or physically connected to form a large array. In the U.S. for example, marine cage systems for salmonids typically consist of 10 to 50 cages moored together in a single large array. Salmonid production at most commercial facilities will reach at least 25 t/yr. Production tends to be greater at marine farms, and at the largest production facility may exceed 500 t/yr.

More than 90% of the world's farmed salmonids are reared in marine cage systems. However, given the notoriously broad salinity tolerance of salmonids and the diversity of culture practices throughout the world, generalities are difficult. In many areas of the world, freshwater cage culture comprises a significant proportion of salmonid

TABLE 7

Flow in the receiving river per ton (t) of fish produced in relation to potential environmental effects (from Alabaster 1982).

| River flow ($l \cdot s^{-1} \cdot t\ fish^{-1} \cdot yr^{-1}$) | Expected environmental effect |
|---|---|
| > 13.4 | possible mild enrichment |
| < 13.4 | possible sewage fungus |
| < 8 | low dissolved oxygen, solids accumulation, sewage fungus |
| < 5 | downstream fisheries adversely affected |

production. In Scotland for example, 14% of the rainbow trout are reared in marine cages, 42% of the trout production comes from freshwater cages, and the remainder comes from freshwater ponds and tanks (NCC 1990). In Finland, about 85% of farmed rainbow trout comes from brackish-water cages, with the remainder coming from freshwater cages (ICES 1989). In general, there has been a recent tendency in many countries to shift trout production from freshwater systems into marine cages.

### *Waste Products and Loadings*

Cage culture typically requires 1.3-2 kg of feed to produce 1 kg of fish (wet weight basis). With a nitrogen content in the feed of 6-8% and the nitrogen content of a salmonid typically about 2.5-2.7% (Wallin and Håkanson 1991), it is clear that a substantial portion of the nitrogen entering the cage system in the form of feed does not leave as fish biomass at the time of harvest. Approximately 80 kg of nitrogen are released to the environment for every ton of fish produced (Ackefors and Enell 1990). A comparable analysis for phosphorus indicates that the feed concentration is typically 1-1.5% and salmon flesh is 0.4-0.5%. About 10-20 kg of phosphorus are released to the environment for every ton of fish produced (Enell and Löf 1983; Ackefors and Enell 1990; Holby and Hall 1991).

As a result of several flux studies at cages in freshwater (Penczak et al. 1982; Enell and Löf 1983) and marine waters (Holby and Hall 1991), considerable information is now available on the fate of nitrogen and phosphorus originating from salmonid cage-culture. Only about one-fourth of the nitrogen supplied to caged salmonids is removed with the harvest (Figure 3). About one-half of the nitrogen leaves the cages in a dissolved form, principally ammonia. Another one-fourth is incorporated in the solid wastes and deposited in the sediments, although a substantial portion of this particulate nitrogen will

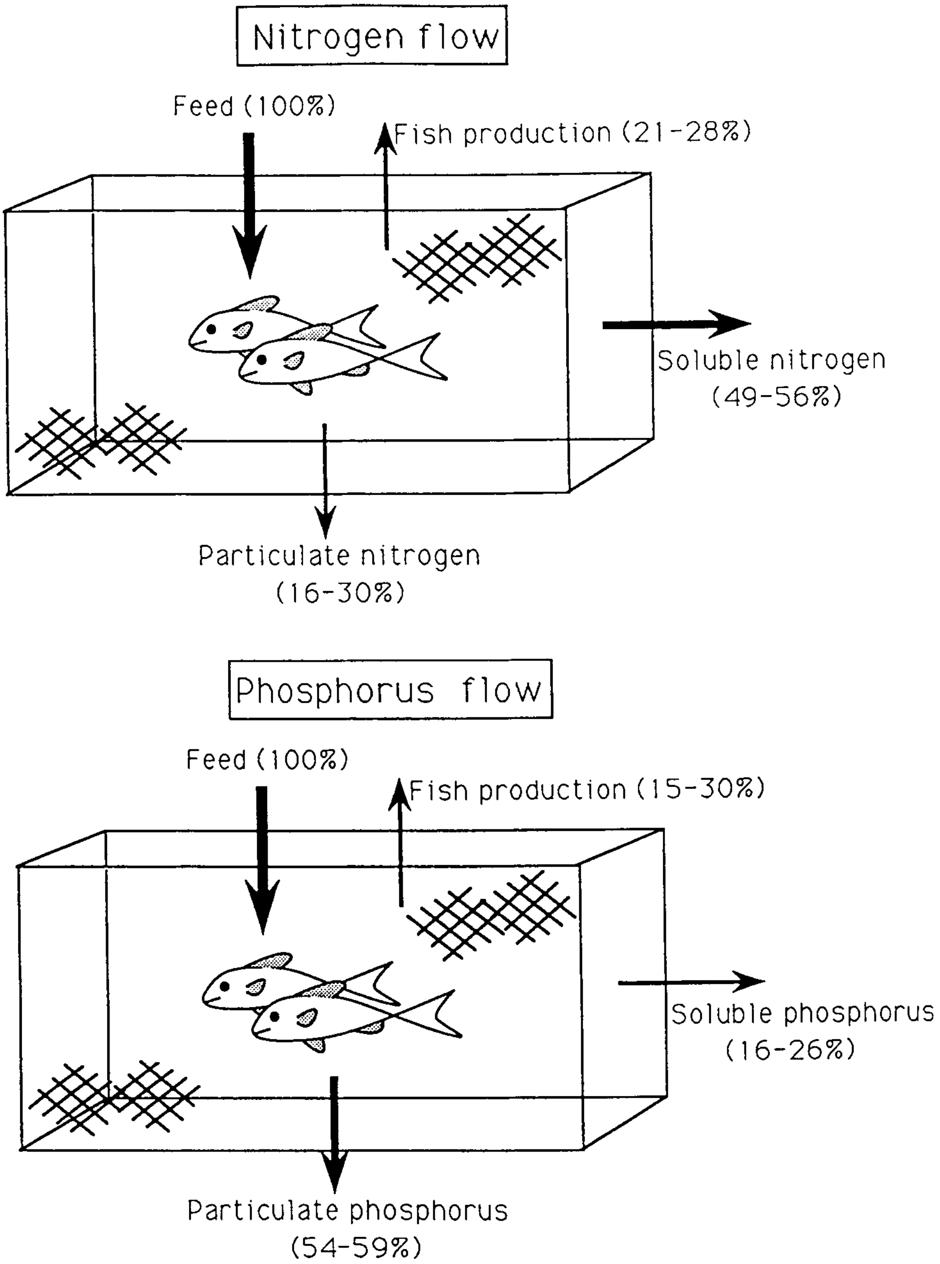
Nitrogen flow
Feed (100%)
Fish production (21-28%)
Soluble nitrogen (49-56%)
Particulate nitrogen (16-30%)
Phosphorus flow
Feed (100%)
Fish production (15-30%)
Soluble phosphorus (16-26%)
Particulate phosphorus (54-59%)

FIGURE 3. Nitrogen and phosphorus flow through salmonid cage culture assuming 100% of each nutrient is provided in the feed. Based on data in Phillips and Beveridge (1986), Ackefors and Enell (1990) and Holby and Hall (1991).

ultimately be released to the water column as ammonia as decomposition of the organic-rich wastes proceeds.

As is the case for nitrogen, about one-fourth of the phosphorus supplied to cage-cultured salmonids in the feed is removed with the harvest. However, phosphorus is dramatically different from nitrogen in that only about one-fourth enters the environment in the dissolved phase. The majority of waste phosphorus is deposited in the sediments beneath the farm. The proportion of sedimentary phosphorus later introduced into the water column has been reported to be only 4-8% in some cases (Holby and Hall 1991), but may reach 50% of the sedimentary phosphorus at other farms (Enell 1987). Release of phosphorus from enriched sediments beneath cages is accelerated under anaerobic conditions (Enell and Löf 1983).

### *Environmental Impacts*

*Benthic Effects.* The sediments beneath salmonid cage-culture facilities are frequently subject to high organic loading from deposition of fish feces and waste feed. Sedimentation rates beneath cages are commonly 2-20 times greater than in reference areas (Pease 1977; Enell and Löf 1983; Hall and Holby 1986; Weston and Gowen 1988; Kupka Hansen et al. 1991; Ye et al. 1991), but in some freshwater lakes sedimentation rates may reach 100-200 times background rates (Merican and Phillips 1985; Phillips et al. 1985*a*). This deposition changes the chemical and biological characteristics of the sediment, and often its physical appearance. Sediments beneath cages are often typified by waste feed pellets scattered on the sediment surface, bacterial mats at the sediment-water interface, gas ebullition in some cases, and a layer of flocculent, organic-rich material overlying the natural sediments. Although usually only a centimetre or two in thickness, this organic-rich layer may, in worst cases, reach 30-40 cm in thickness (Ervik et al. 1985; Kupka Hansen et al. 1991). In general, deposition is greatest at sites where water currents are reduced such as in lakes or small, nearly-enclosed, marine embayments. At exposed marine sites deposition may be negligible.

At all sites where solid waste deposition has been reported it is a localized occurrence. Visible accumulation and accompanying chemical and biological impacts are greatest directly under the cages, and commonly extend out 15-50 m from the perimeter (Enell and Löf 1983; Doyle et al. 1984; Brown et al. 1987; Weston and Gowen 1988; Lumb 1989; Aure et al. 1988). Recent evidence from stable carbon isotope studies (Ye et al. 1991; Kupka Hansen et al. 1991) suggest that cage-derived carbon may be detectable for a considerable distance from a farm, but that sedimentation rates causing significant biological impacts are much smaller in spatial extent. Investigations at an Australian marine cage farm (Ye et al. 1991) indicated that nearly 80% of the carbon directly beneath the cages had an isotopic signature typical of salmonid feed and feces. At a distance of 60 m about 40% of the carbon was farm-derived. Impacts on macrofaunal communities, however, were measurable only 10-30 m from the cages.

Sediments enriched by salmonid feed and fecal matter exhibit the same chemical changes typical of organic enrichment in general. Sediment oxygen consumption is elevated, usually by about an order of magnitude (Enell and Löf 1983; Hall et al. 1990; Kupka Hansen et al. 1991). Sediment reduction-oxidation potentials are more negative, and the aerobic portion of the sediment column is shallower (Brown et al. 1987). Anaerobic microbial degradation of organic matter results in increased fluxes of methane and, in marine waters, hydrogen sulphide from the sediment to the overlying water (Gowen et al. 1991). These releases may be visible as bubbles comprised mostly of methane and potentially with lesser amounts of carbon dioxide (0-30%) and hydrogen sulphide (0-2%) (Håkanson et al. 1988; Samuelsen et al. 1988; Hall et al. 1990). Nitrogen is released from enriched sediments principally as dissolved organic compounds and ammonium; rates of nitrification (conversion of ammonium to nitrite and nitrate) and denitrification (nitrate to elemental nitrogen) are negligible due to the absence of oxygen in enriched sediments (Kaspar et al. 1988; Kupka Hansen et al. 1991). Phosphorus content of fish feed and fecal matter is usually far above that of natural sediments, and thus may be a good indicator of farm influence (Holby and Hall 1991). Phosphorus flux to the overlying water has been reported to be about 40 times greater than in reference areas and is usually accelerated under anoxic conditions (Enell and Löf 1983; Holby and Hall 1991; Kupka Hansen et al. 1991; Kelly 1992).

Benthic infaunal community composition may be dramatically altered as a result of the low dissolved oxygen and high sulphide concentrations of interstitial water in sediments impacted by cage culture. Study of these impacts have largely been limited to marine systems (*see* review by Gowen et al. 1991), but benthic communities beneath cages sited in freshwater environments should exhibit the same general structural and functional changes as their marine counterparts.

The benthic impacts of cage culture typically include the elimination of most macrofaunal species. A reduction in areal species richness in the sediments under and around salmonid cage-culture sites has proven to be a consistent and reliable indication of impact (Figure 4). In most cases this reduction in species number is accompanied by a peak in macrofaunal density reflecting the prevalence of one or two opportunistic species, often found at densities of 10,000 individuals$\cdot$m$^{-2}$ or more. At marine salmon farms the most commonly reported opportunistic organisms have been polychaetes of the *Capitella capitata* species complex (Pease 1977; Tsutsumi and Kikuchi 1983; Aure et al. 1988; Weston and Gowen 1988; Rosenthal and Rangeley 1989; Weston 1990). In brackish waters chironomids predominate beneath the cages (Laurén-Määttä et al. 1991).

As is typical for organic enrichment in general, the enrichment of sediments resulting from cage culture selectively eliminates the large, deep-burrowing members of the macrofaunal community (e.g., some molluscs and the larger polychaetes). Their disappearance reduces the proportion of community biomass deep within the sediment column, and shifts the biomass profile towards the sediment-water interface (Weston 1990). The elimination of these larger species also contributes to a reduction in

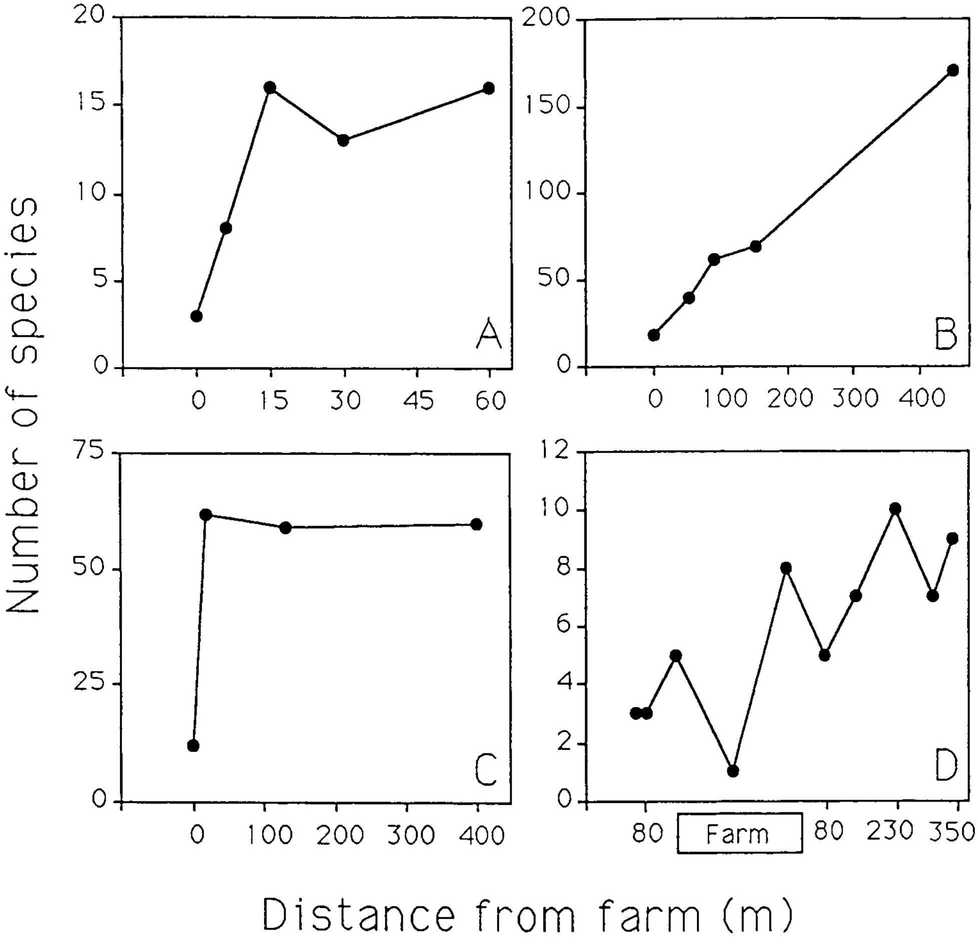

FIGURE 4. Trends in number of species per sample with distance from four salmonid cage culture sites. (A) Puget Sound, northwestern U.S. (Weston and Gowen 1988); (B) Puget Sound, northwestern U.S. (Weston 1990); (C) Loch Spelve, Scotland (Brown et al. 1987); (D) Kaurissalo, Finland (Laurén-Määttä et al. 1991).

parameters such as average individual size within the community. Conversely, those animals capable of tolerating the enriched conditions may actually exhibit an increase in body size because of an enhanced food supply. Individuals of *Capitella capitata*, for example, may be 3-5 times larger in the enriched area near the cages than individuals from populations only a few hundred meters distant (Tsutsumi 1990; Weston 1990).

As noted above for benthic effects in general, effects on macrofaunal community structure are highly localized around the farm site. The greatest spatial extent of impact was reported at a very large (620 tons annual production) marine cage site where dramatic macrofaunal community changes were evident up to a distance of 45-90 m, and more subtle effects extended to at least 150 m (Weston 1990). Although the benthic impacts of any one farm are localized, impacts of many farms may be cumulative. When a substantial portion of a water body is dedicated to salmonid cage culture, benthic community alteration may be evident over broad areas of the bottom (O'Connor et al. 1989).

While the benthic impacts of cage culture can be minimized, mortality of the more sensitive macrofaunal species and a predominance of opportunists are probably unavoidable except perhaps in very exposed off-shore areas. At some farms, however, enrichment can be so great as to eliminate even the opportunistic species, resulting in azoic conditions beneath the cages (Brown et al. 1987; Rosenthal and Rangeley 1989; Kupka Hansen et al. 1991; Laurén-Määttä et al. 1991). Such conditions would indicate poor site selection and an imminent threat to viability of the farm itself. Perhaps it is not entirely coincidental that at most farms where azoic conditions have been reported, the sites have since been abandoned [sites investigated by Brown et al. (1987), Rosenthal and Rangeley (1989), and Kupka Hansen et al. (1991)]. The most direct and obvious threat to the farm from such gross sediment enrichment is the toxicity of sulphides emanating from the sediments, but there many be other reasons to avoid such sites. The irrigation and burrowing activities of *C. capitata* are known to hasten the degradation of sediment-associated contaminants such as aromatic hydrocarbons (Gardner et al. 1979). For those antibiotics such as oxytetracycline and oxolinic acid that are lost from the sediments only by diffusion to the overlying water, the elimination of *C. capitata* or similar species may dramatically increase the persistence of the antibiotic residues, potentially increasing the selective pressure for emergence of antibiotic-resistant strains.

*Hypernutrification and Eutrophication.* The release of nitrogen and phosphorus from salmonid cage culture has led to concerns that the industry may contribute either to hypernutrification (increased nutrient concentrations in receiving waters) or eutrophication (increased primary productivity). Most authors have regarded the potential for direct toxicity from ammonia output to be slight, but the potential for nutrient enhancement of primary productivity has attracted considerable attention. This concern has been particularly acute in those countries surrounding the Baltic Sea. Although fish farming contributes a very small fraction of the total nutrient load to the Baltic, eutrophication of the Sea as a whole and concern for more localized effects has led to considerable effort in quantifying, modelling and controlling nutrient releases from cage culture (Håkanson et al. 1988; Ackefors and Enell 1990; Mäkinen 1991*b*).

It is not unusual to measure elevated nutrient levels in the immediate vicinity (tens of metres) of cage culture facilities. At several Norwegian marine cage culture facilities ammonia concentrations in surface water near the farms were often 2-10 times greater than those in reference areas (Ervik et al. 1985; Aure et al. 1988). Ammonium concentrations near a Scottish cage site were elevated 3- to 6-fold at the farm, and concentrations returned to background levels at the next closest station at 1 km distance. Other investigators of marine cage farms have reported either no measurable increase in nitrogen or phosphorus concentrations, or an increase only under unusual circumstances (Hagstrom and Larsson 1982; Kaspar et al. 1985; Müller-Haeckel 1986).

Cage culture in freshwater environments is also possible with no measurable increase in nutrient concentrations, or with only very localized increases (Phillips 1985). There are, however, several instances where farms in relatively small water bodies have increased nutrient concentrations to the point where hypernutrification of the entire water body is suspected. Nitrate and ammonia levels in a small freshwater loch in Scotland increased after the initiation of trout cage culture (Phillips 1985). Annual production of 30 tons of trout in a 4-km$^2$ lake in Poland increased nitrogen content of the epilimnion by 6-23% and phosphorus content by 15-29% (Korzeniewski and Salata 1982). Production of about 45 tons of trout in both the Uniesc and Skotawa Rivers in Poland resulted in dramatic changes in water chemistry (dissolved oxygen, BOD) and nutrient levels (Korzeniewski et al. 1982). Increased nutrient levels persisted for at least 8 km downstream of the farm sites in both rivers (Figure 5). In these cases trout were held in weirs rather than cages *per se*, but the absence of waste recovery or treatment would make the environmental effects essentially identical.

Whereas it has been repeatedly demonstrated that cage culture may contribute to hypernutrification, whether localized or widespread, documentation of aquaculture-related impacts on eutrophication has been difficult, particularly at marine sites. Localized or ephemeral increases in nutrient concentrations at marine culture sites have not contributed to measurable eutrophication. There generally have been no measurable effects of marine culture on phytoplankton cell density, phytoplankton biomass, or primary productivity (Pease 1977; Müller-Haeckel 1986; Gowen et al. 1988). In freshwater culture there have been more frequent reports of increased primary productivity attributable to cage culture (Kilambi et al. 1976; Hays 1980). Stirling and Dey (1990) studied a small (71 ha) Scottish loch supporting 200-300 tons of rainbow trout production per year and reported the presence of microalgal species typical of nutrient enrichment, a shift from a nutrient-limited to light-limited phytoplankton community, and a general eutrophication of the entire loch ecosystem. They attributed these changes to salmonid farming, but such reports are the exception. There are three principal reasons for the limited evidence of eutrophication. First, while many investigators have measured nutrient levels in waters surrounding cage sites, fewer have measured primary production. In many of the cases where hypernutrification has been greatest and eutrophication is suspected (Penczak et al. 1982 and the Polish examples cited above), the investigators failed to measure the response of the plankton community to the elevated nutrient levels. Secondly, while ammonia and phosphates released from aquaculture are readily assimilated by phytoplankton, increased nutrient concentrations may be inadequate to stimulate algal growth if other factors required to support growth (e.g., light, trace

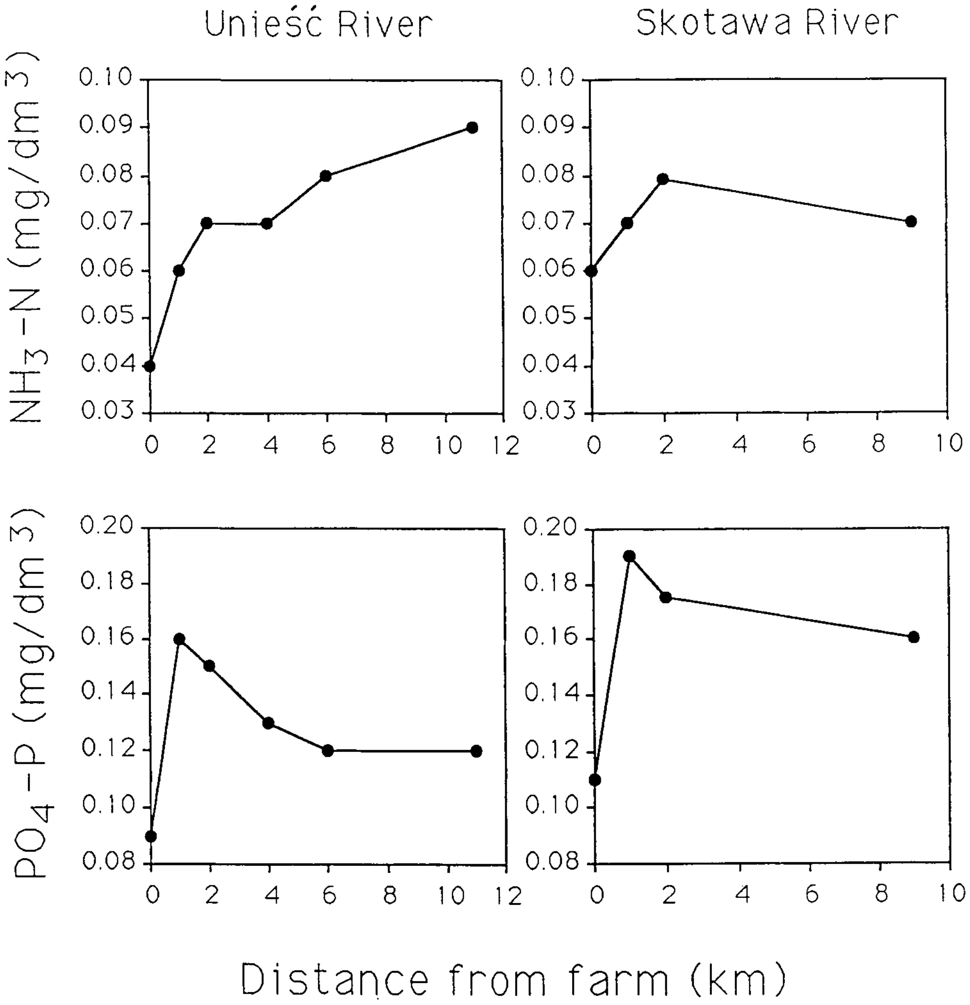

FIGURE 5. Concentrations of ammonia and phosphate downstream of salmonid culture sites in two Polish rivers (from Korzeniewski et al. 1982).

elements) are unavailable. Finally, a significant time period (hours to days) is required to convert the elevated nutrient levels to algal biomass. Except under stagnant conditions, this time period would be sufficient to dilute the nutrient-enriched waters with surrounding unenriched waters, making the increased primary productivity difficult or impossible to measure.

In summary, while salmonid cage culture may contribute to hypernutrification, evidence of eutrophication is very limited. In most coastal marine waters the release of nutrients from cage culture is likely to be of negligible consequence. Nevertheless, the potential for impact exists and merits consideration in farm siting.

Within confined embayments nutrient loading may be cause for concern if a substantial portion of the embayment is dedicated to culture. The potential for eutrophication is greatest in freshwater environments, particularly if the water body is small and the water column is shallow or seasonally stratified.

*Dissolved Oxygen.* Salmonid culture can deplete dissolved oxygen concentrations in both surface waters (primarily due to fish respiration) or near-bottom waters (due to BOD of solid wastes). Localized depletion of surface water dissolved oxygen concentrations by about 2 mg/l have been reported around marine cage-culture sites in the U.S. (Pease 1977) and Japan (Kadowaki and Hirata 1984 - Figure 6). In a survey of eight farms in freshwater Scottish lochs, an average depression in dissolved oxygen of 0.7 mg/l relative to reference areas 150 m from the cages was reported (Phillips 1985). Such localized depressions in surface water dissolved oxygen are unlikely to be of major ecological significance. Large active fish such as salmonids are among the most sensitive animals to oxygen depletion, and would be stressed if dissolved oxygen levels dropped below about 5 mg/l for extended periods. Should fish respiration decrease dissolved oxygen concentrations to biologically limiting levels, the fish themselves would be the first to suffer the consequences and standing stock within the farm would decrease to below sustainable levels.

Decreases in bottom-water dissolved oxygen because of the BOD of feces and waste feed could have environmental ramifications without immediate adverse consequences to the cultured fish themselves. Decreases in bottom-water dissolved oxygen levels of 2-5 mg/l have been reported beneath cages (Pease 1977; Brown et al. 1987), but generally the effects are limited to benthic community impacts directly under or within very close proximity of the farm. Effects may be of greater ecological significance when the cages are sited over stagnant bottom waters isolated by bathymetric features or water column stratification. In a small, stagnant, Scottish loch the BOD of farm wastes coupled with sedimentation of algal biomass due to eutrophication was blamed for a hypoxic event of atypical severity and duration (Phillips 1985). Such concerns have led authorities in some countries to recommend cage siting in the shallow portion of lakes or marine fjords, avoiding sites over deep hypoxic waters (Sweden — Thorslund 1980; Norway — Aure and Stigebrandt 1989).

*Wild Fish Populations.* Several authors have reported changes in fish populations around cages due to the physical cage structure (Beveridge 1987) and abundance of both natural (Kilambi et al. 1976) and uneaten artificial feed (Phillips et al. 1985*b*). At freshwater cage sites in Scotland, brown trout (*Salmo trutta*), Arctic charr (*Salvelinus alpinus*), and rainbow trout have all been found to congregate around salmonid cages.

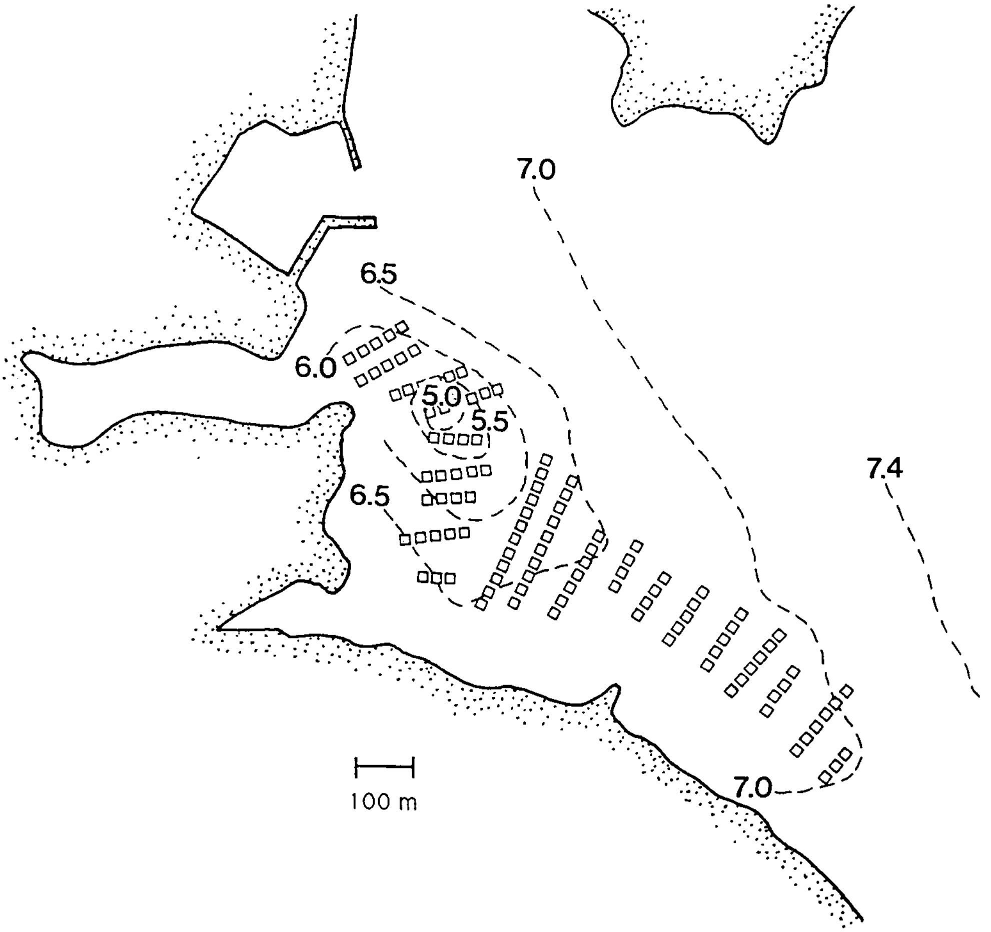

FIGURE 6. Dissolved oxygen concentrations in surface waters surrounding cages containing 400 tons of yellowtail (*Seriola quinqueradiata*) and sea bream (*Acanthopagrus australis*) in Usui Bay, Japan. Dissolved oxygen concentrations were measured on three occasions; Figure 6 shows the greatest depression of dissolved concentration as measured immediately after feeding (from Kadowaki and Hirata 1984).

Fish production may be enhanced as a result of cage culture, with increased growth of native and introduced fish being recorded following introduction of cages (Kilambi et al. 1978; Phillips et al. 1985*b*). The observed improvements in growth may be due to fish feeding on uneaten pellets or some stimulation of natural productivity. Hays (1980), Loyacano and Smith (1976) and Kilambi et al. (1978) noted that many species of predatory and non-predatory fish were caught in greater numbers adjacent to channel catfish (*Ictalurus punctatus*) and rainbow trout cage farms. Kilambi et al. (1978) also found that the survival as well as growth of certain wild fish species was improved as a result of the introduction of cage culture.

One potential problem is that eutrophication can lead to changes in community structure and the loss of sensitive species. Penczak et al. (1982), for example, expressed concern that eutrophication caused by cage culture in Polish lakes would result in irreversible loss of native coregonid fisheries. Evidence from other studies shows that eutrophication can be detrimental to fish populations and that salmonids are particularly vulnerable (Colby et al. 1972; Maitland 1984). The response of salmonid fish communities to eutrophication is a complex issue, as eutrophication can lead to changes in food availability, algal blooms and increases in the number of microorganisms (Bird and Kalff 1984), all of which may increase susceptibility to disease (Grimaldi et al. 1973).

Cages are more susceptible than other fish-farming systems to fish losses through storm damage, collisions with boats, predator attacks, vandalism and other accidental losses sustained during handling and net changing (Beveridge 1987). Numerous losses have been reported in recent years, including single losses of 100,000 fish (Maitland 1989) and 185,000 fish (Anon. 1989) and 20-30 tons of Atlantic salmon (Hansen et al. 1987). Estimates from freshwater lakes indicate that escapes may account for 2.5-5.0% of caged rainbow trout annual production (Penczak et al. 1982; Phillips et al. 1985*b*). The scale of losses from salmon cage farming is such that farmed Atlantic salmon are becoming an important component of captured fish stocks. For example, in Norway, 25% of fish ascending rivers are thought to have escaped from fish farms (NASCO 1989). In Scotland, the proportion of farm fish at netting stations on the north and west Scottish coasts has increased from below 2% in the early 1980s to 6% in 1989 (Scottish Office Agriculture and Fisheries Department, pers. comm.). Farmed Atlantic salmon have also been caught in the high-seas fishery north of the Faroe Islands (Craik et al. 1987).

Behavioural interactions and interbreeding between farm and native stocks are of particular concern because of the potential for loss of native stocks and genetic diversity (NASCO 1989; NCC 1990). As far as is known, salmonid farm escapees tend to fare poorly in competition with native stocks (Duncan 1991). However, the potential impacts associated with the release of large numbers of farm stock could be significant in some locations, particularly in small spawning streams and rivers with a limited fish-carrying capacity (Maitland 1989). Several authors have demonstrated that there are genetic differences between wild and farmed Atlantic salmon and that wild stocks of salmon from adjacent rivers and even tributaries of the same river are genetically different (Verspoor 1989). However, the significance of the observed genetic variation and the extent and impact of any interbreeding has not been established.

The effect of salmonid farming on disease in wild stocks has become a controversial issue in recent years (NCC 1990). Movements of farmed fish have certainly led to the transfer of microbial pathogens (e.g., Enteric Redmouth Disease bacterium *Yersinia ruckeri* and Infectious Hematopoietic Necrosis virus, both from North America to Europe) and parasites. However, there is less evidence that introduction of such pathogens has led to clinical disease in wild stocks. The possible exceptions are the spread of furunculosis throughout the U.K. in the early 1900s and introduction of the monogenean *Gyrodactylus salaris* into Norwegian rivers where it has been reported to cause massive mortalities of young salmon (Maitland 1989) (the relationship between the decline of salmon stocks and the epizootic of *G. salaris* is still uncertain —*see* Halvorsen and Hartvigsen 1989). Most disease organisms present in farmed stocks are also present in wild stocks, but farmed fish are more susceptible to outbreaks of clinical disease because of the higher levels of stress, high stocking densities, and more rapid transmission of disease organisms that may be found in the culture environment. There is no evidence as yet that outbreaks of disease in farmed fish raise pathogen levels in the wild fish to a level where the latter suffer clinical disease (Weston 1986; NCC 1990). Even so, care is very much warranted through appropriate screening and quarantine procedures to minimize risks.

*Chemotherapeutants.* Because of the unconfined nature of cage-culture systems all chemotherapeutants except those assimilated by the cultured fish are released to the surrounding environment, either by direct release following immersion therapy or from urine, feces, and uneaten feed. There is a risk that chemotherapeutants could have unintended adverse impacts on non-target species in the surrounding area, and dilution generally serves as the only form of protection. Unfortunately, however, many chemicals have been approved by regulatory agencies and are in wide use with little consideration given to potential environmental effects.

Among those chemicals used in immersion treatments in cage culture, trichlorphon (Neguvon) and dichlorvos (Nuvan) have received the most attention, although, even for these chemicals, the data are very limited. Although use has declined considerably in recent years, trichlorphon or its degradation product, dichlorvos, have been widely used in marine cage culture throughout the North Atlantic for the treatment of ectoparasitic salmon lice. Treatment is normally done by surrounding the cage with a plastic or canvas skirt or bag during the period of treatment, and the skirt or bag is removed after completion of chemotherapy. Dichlorvos is used at a concentration of 1 ppm in Scottish cage culture; trichlorphon is used at concentrations of 10 to 300 ppm in Norwegian cage culture. Non-target organisms have been found to be far more sensitive to both compounds than the cultured fish. A 1-h exposure to 1 ppm dichlorvos is toxic to larval lobsters, adult lobsters, zooplankton and phytoplankton (Cusack and Johnson 1990). Concentrations of trichlorphon between 0.5 and 10 ppm are toxic to adult lobsters after exposure periods of 18 and 6 h, respectively (Egidius and Møster 1987). The potential therefore exists for immersion treatment to adversely affect non-target invertebrates in the vicinity of the cage system, and trichlorphon treatment at one Norwegian salmon farm has been blamed for mortality of nearby lobsters (Egidius and Møster 1987). A similar study of dichlorvos treatment at a Canadian farm failed to find an effect on nearby caged lobsters (Cusack and Johnson 1990). The potential for environmental harm, the paucity of information on fate and effects, misuse of trichlorphon and dichlorvos by some farmers, and inadequacies of regulatory control in some countries have resulted in considerable controversy and calls for the prohibition of these substances in salmonid culture (Ross 1989).

Among the chemotherapeutants administered as feed additives, the antibiotics have raised considerable concern given the quantities used in some countries, minimal knowledge of environmental fate processes, and extremely limited information regarding environmental effects (Brown 1989; Lunestad 1991). It is apparent that a considerable portion, and in some cases the vast majority, of the antibiotics provided to the cultured fish are released to the environment. Feed wastage is likely to result in a loss to the environment of 1 to 40% of the antibiotic provided, particularly as diseased fish often exhibit a reduced appetite. Much of the antibiotic ingested by the fish may pass through the digestive tract without being assimilated and is released to the environment via the feces. Approximately 92% of the oxytetracycline ingested by rainbow trout passes through the gut without being assimilated; 62-86% of oxolinic acid is unassimilated (Cravedi et al. 1987). Thus the majority of these and other antibiotics are likely to reach the sediment beneath the cage-culture site.

It is becoming apparent that the length of time antibiotics persist in sediments is highly dependent upon microbial degradation and sedimentation rates. The antibiotic furazolidone is readily metabolized by sedimentary bacteria and has a sediment half-life of only 18 h (Samuelsen 1991). On the other hand, the more widely used antibiotics, oxytetracycline and oxolinic acid, are lost from sediments only by diffusion into the overlying water and therefore may persist in sediments for months or even years. Reported sediment half-lives for oxytetracycline range from 1 wk to 60 wk (Jacobsen and Berglind 1988; Samuelsen 1989; Bjorklund et al. 1990), with persistence greatest under conditions of rapid sedimentation.

Virtually nothing is known about the effects, if any, antibiotics persisting in sediments may have on the environment, but three concerns have arisen and are now the subject of investigations in several countries. First, antibiotics fed to cattle can alter the biodegradability of their manure (Elmund et al. 1971), so it seems possible that aquacultural antibiotics could have similar effects on organic matter degradation rates in aquatic sediments. Sediments beneath cage-culture sites are typified by intense microbial activity, and the addition of antibiotics could alter rates of a wide variety of microbially-mediated biogeochemical processes such as methanogenesis and sulphate reduction. In laboratory experiments oxytetracycline has been shown to affect rates of ammonification, nitrification and thiosulphate oxidation by marine bacteria (Carlucci and Pramer 1960). Preliminary experiments seem to indicate a decrease in sediment bacterial densities following chemotherapy with oxytetracycline (Samuelsen et al. 1988) or oxolinic acid (ICES 1990).

Secondly, the persistence of antibiotics in the environment could foster the development of antibiotic resistance in aquatic bacteria, potentially compromising the efficacy of later chemotherapy. Antibiotic therapy is known to increase the proportion of antibiotic-resistant bacterial strains in effluent from land-based salmonid culture sites (Austin 1985), and the same effect may occur in the sediments beneath cage-culture facilities. The few published studies on this subject have found higher levels of oxytetracycline resistance in bacteria beneath farms subject to antibiotic treatment than in otherwise comparable reference areas (Torsvik et al. 1988; Husevaag et al., in press).

Finally, antibiotics released to the environment from salmonid culture could be assimilated by wild fish or demersal invertebrates such as crabs or molluscs, and thereby provide an unwanted dietary intake of antibiotics to human consumers. Fish farms in both fresh and marine environments attract large populations of escaped and wild fish (Carss 1990), making them popular areas for recreational fishing. Whether dietary consumption of antibiotics through recreational fishing is significant has not been investigated, but it is known that wild fish and mussels in the vicinity of Atlantic salmon cages have measurable concentrations of oxytetracycline and oxolinic acid during chemotherapy of the caged fish (Møster 1986; Bjorklund et al. 1990; Lunestad 1991).

### *Impact Mitigation*

*Holding Capacity.* In the context of aquaculture and the environment, holding capacity is used to denote the maximum aquaculture production attainable in a water body without eliciting an unacceptably high degree of environmental change. The more commonly used term "carrying capacity" is defined as the maximum production sustainable in a water body because of limited resources (e.g., oxygen or food availability) (Rosenthal et al. 1988).

Holding-capacity models for aquaculture are best developed in freshwater systems. Several models for determining the response of standing waters to phosphorus loading are available, including dynamic models and empirically derived mass balance models. Dynamic models consist of a series of interrelated differential equations which attempt to describe the biological, chemical and physical interactions that govern algal growth (Kamp-Nielsen 1985). Such models require detailed data inputs and tend to be site-specific. Mass-balance models are based on correlations between phosphorus loading and indicators of trophic state (Vollenweider 1968; Dillon and Rigler 1974; OECD 1982). Such models have been widely applied to lake management because of simpler data requirements, and have been modified for use in cage fish farming (Beveridge 1984; Phillips 1985; Carlsson 1988).

The Vollenweider (1968) model, modified by Dillon and Rigler (1974) and adapted for cage culture by Beveridge (1984), is a simple mass-balance equation with components for the input of phosphorus; a sedimentation coefficient, which estimates the loss of phosphorus to the sediments; and water flow, which determines the amount of phosphorus lost via the outflow. The equation for the model is:

$$[P] = (T_w \cdot L \cdot (1\text{-}R)) / zt \qquad (2)$$

where $[P]$ = predicted lake phosphorus concentration (mg $m^{-3}$); $T_w$ = water residence time (yr); $L$ = areal loading rate (mg $P{\cdot}m^{-2}{\cdot}yr^{-1}$); and $z$ = mean depth (m). $R$ is the sedimentation coefficient, commonly calculated using the equation from OECD (1982) where $R = 0.426\ e^{(-0.271 \times qa)} + 0.574\ e^{(-0.00949 \times qa)}$ where qa is the hydraulic load, $z/T_w$.

For cage farms, the increase in phosphorus due to fish culture must be added to the background concentration to give a final predicted concentration. In principle, a mass-balance approach should account for all the routes by which phosphorus compounds are transported through a lake system. In practice, however, vagaries exist in the application of such models, usually as a result of three major problems: (1) incomplete budgeting of catchment phosphorus sources; (2) effects of climatic and limnological conditions; and (3) incorrect application of model parameters (*see* detailed discussion in NCC 1990). Once total phosphorus is predicted through application of the Dillon and Rigler model, it must be related to trophic state (e.g., oligotrophic, eutrophic). Several studies have linked total phosphorus concentration to lake trophic status (Reckhow and Chapra 1983*a*,*b*; OECD 1982). Comparison of the predicted total phosphorus concentration with such criteria enables some assessment of the likely impact of fish farm development to be made.

Such models have proved useful in Scotland and are now widely used by pollution control authorities there to determine potential impacts (NCC 1990). However, follow-up water quality monitoring is also recommend to both confirm and (if necessary) refine predictions. Although there are problems, these models have been recognized as a positive step towards the development of more effective tools for environmental impact assessment and management of freshwater lakes for aquaculture.

No holding-capacity models exist for marine waters that are as generally accepted or broadly applicable as the Vollenweider-type models are in fresh waters. There have, however, been two published attempts to quantify holding capacities for salmonid culture in marine or brackish waters; one is based upon nutrient loading and the other upon oxygen consumption of solid wastes. Håkanson and Wallin (1991) and Wallin and Håkanson (1991) have attempted to adapt the Vollenweider approach to 1-14-$km^2$ coastal areas of the Baltic Sea, and to develop "load diagrams" that allow managers to determine the amount of fish production an area can support without substantial environmental change. The sensitivity of an area of concern is determined based upon empirical relationships with coastal morphology, water residence time and sediment type. The load diagrams permit an estimate of the extent of environmental change (as quantified by Secchi disk depth) that would be expected for a given sensitivity and a given nitrogen load from fish culture.

Aure and Stigebrandt (1989) developed a model to predict the holding capacity for cage culture in Norwegian fjords based upon the predicted decrease in oxygen concentrations in basin waters below the sill depth as a result of deposition of farm wastes.

Both of these models were developed for specific environments and have not been shown to be of predictive value elsewhere. The Håkanson and Wallin (1991) model is only intended for use in non-tidal Baltic Sea areas, and would therefore not be applicable in most marine waters. The Aure and Stigebrandt (1989) model is applicable only in silled fjords and has not been tested other than in the few Norwegian fjords for which it was developed. For most marine areas, no holding-capacity models are currently available, but the development of such models is an important long-term goal if trial-and-error growth of the industry is to be avoided.

*Reduction of Waste Loading.* Waste loading from salmonid cage culture can be reduced by manipulating the feed composition and its means of delivery. Considerable progress has been made, particularly in Scandinavia, in reducing the phosphorus content of fish feeds and consequently phosphorus loading to aquatic environments. Similar reductions in nitrogen content appear possible, although it may be more difficult to accomplish these reductions without sacrificing fish growth rates (Sumari 1986; Mäkinen 1991*b*). A detailed discussion on minimization of nitrogen and phosphorus waste by manipulation of feed composition was presented earlier with respect to land-based culture (*see* page 932), and similar considerations would apply to floating cage culture as well.

Feed may be provided to fish by any of four methods: hand feeding, demand feeders (feed release triggered by fish behaviour), automatic feeders (a predetermined amount of feed released at regular intervals), or computerized feeders (feed release programmed according to daily physiological cycles, light regime, or other specific requirements). No comprehensive evaluation of these four techniques with regard to feed wastage has been done. However, it is generally accepted that hand-feeding, although the most labour-intensive, results in the least amount of feed wastage and consequently minimizes release of nutrients and organic-rich solid wastes to aquatic environments. In one study, wastage from hand-feeding of caged Atlantic salmon was 1.4%, whereas automatic feeders wasted 40.5% of the feed supplied (Thorpe et al. 1990).

It may be possible to further reduce feed wastage by manipulating feed density and thus altering settling velocity so as to maximize feed capture time for the caged fish. Extruded pellets have a slower settling velocity than steam pellets. In addition, some cage farmers are employing a floating pellet that is released at the bottom of the cages (Ackefors and Enell 1990). This feed type would allow the culturist to judge feed wastage and could substantially reduce the accumulation of solid wastes beneath the cages.

A wide variety of aquaculture waste-treatment techniques were discussed earlier, and all would be equally effective in treating cage-culture solid wastes as they have been in land-based culture. The efficacy of treatment is, however, made moot by the current unavailability of viable collection technologies. Worldwide, only two approaches to the collection of solid wastes from cages have been employed. Substrate vacuuming has been tried at at least one farm (Rosenthal and Rangeley 1989), but from an ecological perspective may do more harm than good, and in this case at least, the wastes were simply dumped again at some distance from the farm. Several European investigators have experimented with the use of large funnel-shaped devices suspended below the pens to collect feed and feces (Tucholski and Wojno 1980; Enell et al. 1984; Leminen et al. 1986; Solander 1987). These devices have generally proven impractical, have not been adopted in any commercial venture, and, to our knowledge, developmental efforts have ceased. It has proven easier for the culturist and generally acceptable to regulatory agencies to discharge the wastes (albeit usually at a cost to the benthic environment), rather than attempt collection.

The benthic impact of cage culture can be substantially reduced if water currents are adequate to disperse solid wastes over a broad area. If widely dispersed rather than concentrated beneath the cages, the organic-rich wastes may have no measurable impact or a slight stimulatory effect on the benthos due to the enhanced food supply. To date, most attempts to consider natural means of waste dispersal in the selection of cage sites have relied upon rather informal and non-quantitative methods. Lacking any formal guidelines, regulatory officials and the industry have attempted to select culture sites where geomorphology or sediment type would intuitively suggest minimal opportunity for waste accumulation. There have been several recent attempts to formalize this process. Håkanson et al. (1988) suggest empirical and morphometric means to separate bottom areas into erosion, transportation and accumulation categories. The latter would be the most prone to adverse benthic impacts from cage culture, and transportation or erosional bottoms would be preferred for site selection. (It should be noted that some exceptions exist; Aure and Stigebrandt (1989) recommend siting over shallow areas of accumulation to prevent transport of wastes to the deep, hypoxic areas of fjords.) In some areas of the U.S., the selection of sites for cage culture are dictated by interactive depth, current velocity, and farm productivity criteria that strive to maximize the opportunity for horizontal transport of wastes (Rosenthal et al. 1988). Under these guidelines: (1) cages can not be sited where there is less than 6 m between the bottom of the cages and the seafloor, (2) farm productivity and water velocity limits apply when the depth under the cages is between 6 and 18 m, and (3) there are few limitations when depth under the cages exceeds 18 m and mean current velocity at the site exceeds 5 cm/sec. Neither these guidelines nor those of Håkanson et al. (1988) have been rigorously tested and their general applicability is unknown.

There have been several recent attempts to model the dispersal of solid wastes from cage culture (Weston and Gowen 1988; Gowen et al. 1989). These models employ measures of current velocities and estimates of feed and fecal settling velocities to predict the magnitude and areal extent of solid waste deposition. Such models could potentially be of considerable predictive value in site evaluation. At the present time, however, their utility is limited by the inability to extrapolate from a given organic carbon loading to the resulting biological response.

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## Chapter 17

# USES OF BIOTECHNOLOGY TO ENHANCE PRODUCTION

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## INTRODUCTION

As we approach the close of the 20th century, we are witness to a transition from the hunting and capture of salmonids to their semi-intensive and intensive culture. During this transition, biotechnology, in the broad sense, is playing an increasingly important role in the development of sustainable aquaculture (Donaldson 1988). Biotechnology is assisting in this process both by increasing the efficiency of salmonid production systems and by providing means of reducing or preventing reproductive interaction between aquacultured and wild salmonid stocks. For the purposes of this chapter we have adopted a broad definition of biotechnology and have included the application of a number of biological technologies including those based upon recombinant DNA technology, peptide biochemistry, hormone therapies, and chromosome set manipulation.

## INDUCED OVULATION AND SPERMIATION

Salmonids undergo spontaneous final maturation, ovulation and spermiation in captivity, but do not normally spawn in the absence of spawning substrate. While salmonids are normally propagated by *in vitro* fertilization of gametes stripped from fish that have matured spontaneously, there are several circumstances where it is desirable to induce ovulation and spermiation. The first is to obtain gametes up to one month sooner than would otherwise be possible thus enabling earlier initiation of incubation and earlier production of either juveniles and smolts, or production of larger juveniles or smolts.

The second is to facilitate hybridization between strains or species that normally differ in date of maturation. The third is to synchronize ovulation and spermiation to meet production schedules, and the fourth is to ensure that males are spermiating at an optimal level either at the time of egg availability or for cryopreservation of milt. Scientific effort in this area has been intense and the technology has rapidly evolved (Donaldson and Hunter 1983). Initially, whole fresh, or preserved salmonid pituitary preparations containing gonadotropin were used, and these are still used where convenient or appropriate. Later, methods were developed for the preparation of partially purified salmon gonadotropin preparations, e.g., SG-G100 (Donaldson et al. 1972), which were very effective for induction of ovulation in salmon (Jalabert et al. 1978; Hunter et al. 1978, 1981). It is now generally recognized that there are two gonadotropins produced by the salmonid pituitary gland, GtHI and GtHII. Both are involved in gonadal steroidogenesis, the former being produced during gonadal growth and the latter during final maturation when ovarian steroidogenesis switches from estradiol 17$\beta$ to 17$\alpha$, 20$\beta$-dihydroxy-4-pregnen-3-one (17$\alpha$, 20$\beta$ dihydroprogesterone) (Nagahama and Adachi 1985). GtHI and GtHII (maturational gonadotropin) are biochemically and functionally similar to FSH and LH, respectively, in higher vertebrates (Swanson 1991). In recent years, third generation techniques have been developed that either stimulate endogenous gonadotropin synthesis or release, i.e., gonadotropin-releasing hormone (GnRH) analogs (Donaldson et al. 1981*a*) and antiestrogens (e.g., clomiphene citrate, Donaldson et al. 1981*b*), or those which act directly at the level of the ovary, e.g., progesterone derivatives such as 17$\alpha$, 20ß dihydroprogesterone (Jalabert et al. 1978). The physiological mechanisms that regulate reproduction in finfish and the methods of pharmacological intervention that have been developed are shown in Figure 1. A wide range of potent GnRHAs have been developed, several of which have been used for induced spawning in salmonids. Most are based upon the amino acid sequence of mammalian luteinizing hormone-releasing hormone (LH-RH) while others are based upon the amino acid sequence of salmon GnRH or more recently chicken II GnRH (Sherwood et al. 1993). In general, the potent GnRH analogs used to date in fish have been substituted in position 6 with a suitable D-amino acid, and in many the terminal glycine in position 10 has been deleted and replaced with an ethylamide group.

Effective GnRH analogs (GnRHA) that have been used in salmonids include: [D-Ala$^6$, Des-Gly$^{10}$]-LH-RH ethylamide, [D-Ser (t-Bu)$^6$, Des-Gly$^{10}$]-LH-RH ethylamide, [D-Trp$^6$]-LH-RH and [D-Arg$^6$, Des-Gly$^{10}$] salmon GnRH ethylamide. [D-Trp$^6$, Des-Gly$^{10}$]-LH-RH ethylamide appears to be less effective (Haraldsson and Sveinsson 1993). It is expected that analogs of chicken II GnRH will also prove to be effective in salmonids since natural chicken II GnRH is active in fish if the dose is sufficient. One or two doses of 5 - 50 $\mu$g/kg body weight of these hypothalamic peptide analogs is sufficient to induce ovulation or spermiation in most sexually mature teleosts, the dose varying with species and degree of maturity, and the dose interval depending on water temperature, species and degree of maturity.

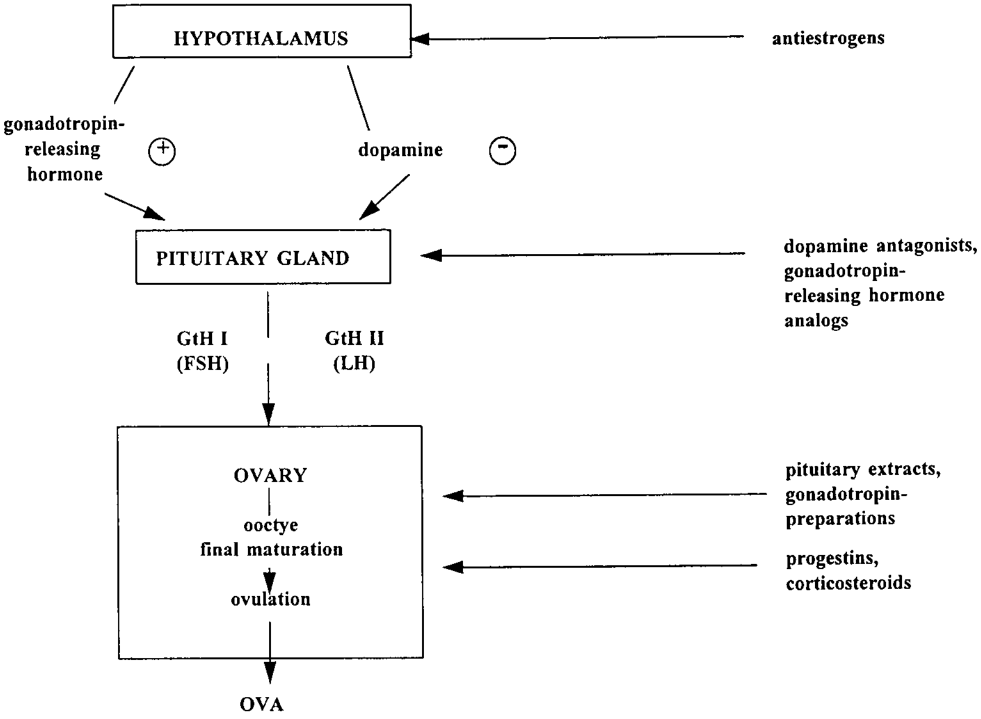
HYPOTHALAMUS
antiestrogens
gonadotropin-releasing hormone
+
dopamine
−
PITUITARY GLAND
dopamine antagonists, gonadotropin-releasing hormone analogs
GtH I (FSH)
GtH II (LH)
OVARY
ooctye final maturation
ovulation
pituitary extracts, gonadotropin-preparations
progestins, corticosteroids
OVA

FIGURE 1. The hypothalamic-pituitary-gonadal axis in fish and methods of pharmacological intervention. Gonadotropin terminology according to Swanson (1991). Modified from Donaldson (1990).

In some non-salmonid species (especially the carp family) there is a strong inhibition of gonadotropin release from the pituitary by the hypothalamic factor dopamine, which prevents GnRHA alone from inducing ovulation (Peter et al. 1988). In these species, it is necessary to inject the fish simultaneously with a dopamine-receptor antagonist such as domperidone. In salmonids successful spawning can be induced by GnRH alone without the use of a dopamine antagonist even though coadministration of such an antagonist can increase plasma type II gonadotropin (GtHII) to a higher level (Van Der Kraak et al. 1986). The GnRHA can be injected into the fish intraperitoneally (i.p.) or intramuscularly (i.m.). It has also recently been demonstrated that GnRH can be absorbed intact from the intestine into the blood in salmonids and that biological activity is retained as assessed by gonadotropin release (McLean et al. 1991). In some species (e.g., sablefish, *Anoplopoma fimbria*) ovulation can be induced by oral administration of GnRHA (Solar et al. 1990). There is one recent report of oral induction of ovulation in a salmonid utilizing GnRHA (Breton et al. 1995).

Over the last decade there has been ongoing research on the development and testing of slow release GnRHA preparations for use in salmonids. Thus, i.p. implantation of a cholesterol pellet containing 38 $\mu$g/kg LHRHA successfully induced ovulation in Atlantic salmon (*Salmo salar*) (Crim and Glebe 1984), and a single injection of [D-Trp$^6$]-LH-RH microencapsulated in a polyglycolic-polylactic biodegradable matrix has been used at a dosage of 12.5 - 50 $\mu$g/kg body weight to induce ovulation in rainbow trout (*Oncorhynchus mykiss*) (Breton et al. 1990). In Atlantic salmon, a single i.m. injection of 75 $\mu$g/kg [D-Ala$^6$, Des-Gly$^{10}$]-LH-RH ethylamide microencapsulated in a biodegradable copolymer of fatty acid dimer and sebacic acid (pFAD-SA) induced ovulation or spermiation after 7-16 days (Myolonas et al. 1995). Biodegradable solid implants containing GnRHA have also been successfully utilized to induce ovulation (Zohar 1988).

In maturing chinook salmon (*Oncorhynchus tshawystcha*), administration of a single polyglycolic-polylactic i.m. implant containing 25 $\mu$g/kg [D-Ala$^6$, Des-Gly$^{10}$]-LH-RH ethylamide 2 weeks before normal ovulation resulted in ovulation in 97% of the fish by day 22 after treatment. In a second group which received a single aqueous i.p. injection at the same dose, 93% of the fish were induced to ovulate by day 37 after treatment. Analysis confirmed that the plasma GnRH concentration in the implant group remained elevated for an extended period (Solar et al. 1995). Survival to the eyed-egg stage was similar in induced and control chinook salmon. Thus, while salmonids close to ovulation can be synchronized with a single aqueous injection of GnRHA (e.g., Van Der Kraak et al. 1987), salmonids that are 2-4 weeks from ovulation require two aqueous injections three or more days apart (e.g., Taranger et al. 1992) or a single controlled-release implant. Spermiation in male salmonids can also be successfully enhanced by a single injection or implant of 25 $\mu$g/kg LHRHA (Solar and Donaldson, unpubl. data).

The main impediment to the use of these synthetic brain peptides is the regulatory aspect, even when they are used in broodstock not destined for human consumption. There is also a need for further research on species-specific dosages and modes of administration. It is possible that more effective GnRH analogs may be developed. However, it is the cost per effective dose that is ultimately the most important factor for aquaculture rather than the specific activity of the peptide per se.

In recent years, the use of sexual pheromones has been explored as a means of regulating reproduction in fishes (Stacey et al. 1993). With further research, this approach may provide a viable technique for synchronizing spawning in salmonids. In male Atlantic salmon parr, the prostaglandins $PGF_{1\alpha}$ and $PGF_{2\alpha}$ increased GtHII concentrations and milt production (Waring and Moore 1995), and exposure of male rainbow trout to female urine has been shown to increase milt production (Vermeirssen and Scott 1995).

## CHROMOSOME SET MANIPULATION

Chromosome set manipulation is a technology that enables the production of salmonids in which all the chromosomes originate from either the male or female parent or in which the number of chromosome sets is increased from the normal pair to either three or four sets (Thorgaard 1983). As its name implies, chromosome set manipulation involves whole sets of chromosomes as opposed to the manipulation of individual genes or chromosomes. In normal salmon and trout, each cell contains two sets of chromosomes (diploid), one originating from the paternal sperm and one originating from the maternal egg. In androgenetic fish, both chromosome sets originate from the sperm and in gynogenetic fish, both chromosome sets originate from the egg. In triploid fish, there are three sets of chromosomes; two sets originate from the egg and one from sperm of the same or a different species. In tetraploid fish, the normal diploid set is doubled to provide four sets of chromosomes. The application of chromosome set manipulation in rainbow trout has recently been reviewed (Thorgaard 1992).

### *Androgenesis*

Androgenesis is a method for producing fish in which all the nuclear genetic information originates from the male parent (i.e., from the sperm) while the mitochondrial DNA is still maternally derived. Androgenesis is induced by gamma or ultra-violet (UV) irradiation of the egg to inactivate chromosomal DNA followed by fertilization with normal sperm. This results in a haploid zygote containing a single chromosome set. The normal diploid state is restored by applying pressure or temperature shock at the first cleavage division so as to duplicate the haploid set and restore the diploid condition. This procedure has been accomplished in only a few laboratories to date, e.g., in rainbow trout (Onozato 1992; Parsons and Thorgaard 1985) and amago salmon (*Oncorhynchus rhodurus*) (Onozato 1993). Recently, Thorgaard et al. (1990) used diploid sperm from tetraploid rainbow trout to fertilize gamma-irradiated ova, thus obviating the need for pressure shock treatment to suppress the first cleavage division. Survival of these androgenetic diploids was much better than those produced using haploid sperm. Fused sperm have also been used to produce diploid androgenetic rainbow trout (Araki et al. 1994). Potential uses of androgenesis include the regeneration of stocks from cryopreserved sperm, the development of identical groups of fish (*see* below), and the production of YY males for the production of monosex male salmonid stocks.

### *Gynogenesis*

Gynogenesis is a method for producing fish in which all the genetic information originates from the female parent, i.e., from the egg. In salmonids, gynogenesis is accomplished by the fertilization of the egg with UV-irradiated sperm (Chourrout 1982;

Ihssen et al. 1990; Johnstone and Stet 1995). The sperm is preferably obtained from a related species that does not produce viable diploid hybrid offspring, thus reducing the chance of obtaining viable, non-gynogenetic offspring as a result of fertilization with incompletely irradiated sperm (Chourrout 1986). Diploidy is usually restored by low (Refstie et al. 1982) or high (Diter et al. 1993; Quillet and Gaignon 1990) temperature, or by pressure shock (Onozato 1984), either shortly after fertilization to prevent separation of the second polar body (meiotic gynogen) or at the time of first cleavage (mitotic gynogen) (*see* Donaldson and Benfey 1987). The former is more easily accomplished than the latter; however, the mitotic gynogens are of interest for the production of clonal lines, despite their generally lower survival and poorer performance (Quillet 1994). Meiotic gynogenesis is also useful for the mapping of genes relative to the centromere (Allendorf et al. 1986).

Gynogenesis may also prove to be a convenient means to produce monosex female salmonid stocks by masculinizing gynogens into phenotypic males which produce monosex female sperm (*see* later section). If gynogenesis is performed using gamma-irradiated sperm, some paternal chromosome fragments can be contributed to the offspring (Disney et al. 1988) resulting in aneuploid individuals.

### *Triploidy*

The induction of triploidy results in salmonids having three sets of chromosomes (Benfey and Donaldson 1988). There has probably been more research and development on induced triploidy than on any other form of chromosome set manipulation in salmonids and their physiology has recently been reviewed (Benfey 1991). Triploidy is achieved by fertilization of normal gametes followed by retention of the second polar body by pressure or temperature shock (e.g., Guoxiong et al. 1989; Teskeredžic et al. 1993*a*), anaesthetic shock (Johnstone et al. 1989) or electrical shock (Teskeredžic et al. 1993*b*). In triploid fish, two chromosome sets originate from the egg and one set from the sperm. Triploids can also be produced by fertilization of gametes from a diploid fish with those of a tetraploid to form interploid triploids (rainbow trout, Myers and Hershberger 1991). Interploid triploids may exhibit better performance than normal triploids and should also ensure the production of 100% triploid populations.

The main production objective of induced triploidy in salmonid aquaculture is inhibition of reproductive development. Triploid female finfish are totally or partially sterile. Thus, ovarian development is inhibited in female triploids and the fish retain the market characteristics of immature fish throughout the year. In male triploid salmonids, testicular development proceeds, but the sperm are aneuploid (Benfey et al. 1986). Aneuploid sperm contains an incomplete set of chromosomes; sperm from a triploid male would be expected to have a ploidy ranging from 1 to 2, averaging 1.5 n.

Triploidy is being used on a commercial scale in the production of non-maturing rainbow trout in North America and Europe and non-maturing Atlantic salmon in Tasmania (Pepper 1991). Triploidy also offers potential for preventing genetic interaction between genetically altered farmed and wild fishes (Devlin and Donaldson 1992; Donaldson et al. 1993*b*). However, in salmonids triploids may not perform as well as diploids especially when grown together (reviewed by Galbreath et al. 1994) and do not exhibit the growth spurt that is typical of normal diploid salmon during sexual maturation. Finally, the production of interspecific triploids may provide hybrids with improved survival or production characteristics, such as disease resistance or seawater tolerance relative to the equivalent diploid hybrid (Parsons et al. 1986; Seeb et al. 1993).

### *Tetraploidy*

The induction of tetraploidy results in salmonids with four sets of chromosomes, and tetraploid fish produce diploid rather than haploid gametes. Tetraploidy has been successfully achieved in few salmonid species to date and significant chromosome damage has been observed (Yamazaki and Goodier 1993). The generally poor survival and performance of tetraploids suggests that the production of tetraploids is not an end in itself, but a means of reaching other biotechnological goals. In the rainbow trout, researchers in both France and the U.S. have successfully produced viable tetraploids using pressure shock prior to first cleavage (Chourrout 1984; Myers et al. 1986). Diploid sperm from tetraploids can be used to directly produce triploids by fertilization of normal ova (Chourrout et al. 1986*b*). The cryopreservation of diploid sperm from tetraploids may also provide a means of recovering valuable fish strains from gene banks through androgenesis (Thorgaard et al. 1990).

### *Cloning of Identical Fish*

The cloning of a teleost was first achieved in the zebrafish (*Brachydanio rerio*) in what is now regarded as a classic study conducted at the University of Oregon (Streisinger et al. 1981). Cloning involves two cycles of gynogenesis, one mitotic and one meiotic in successive generations (Taniguchi et al. 1988) or one cycle of androgenesis and one of gynogenesis (Figure 2).

Researchers in Japan have recently produced small numbers of identical ayu (*Plecoglossus altivelis*) (Taniguchi et al. 1988; Han et al. 1991), salmonids (Onozato 1991), and hirame (*Paralichthys olivaceus*), a species of marine flatfish (Yamamoto 1992). In Europe researchers have cloned another commercially important species, the common carp (*Cyprinus carpio*) (Komen et al. 1991). These early successes suggest that it may be possible in the future to produce groups of completely identical salmonids for aquaculture or to cross different lines of cloned fish to produce identical $F_1$ hybrids (Figure 3). Even

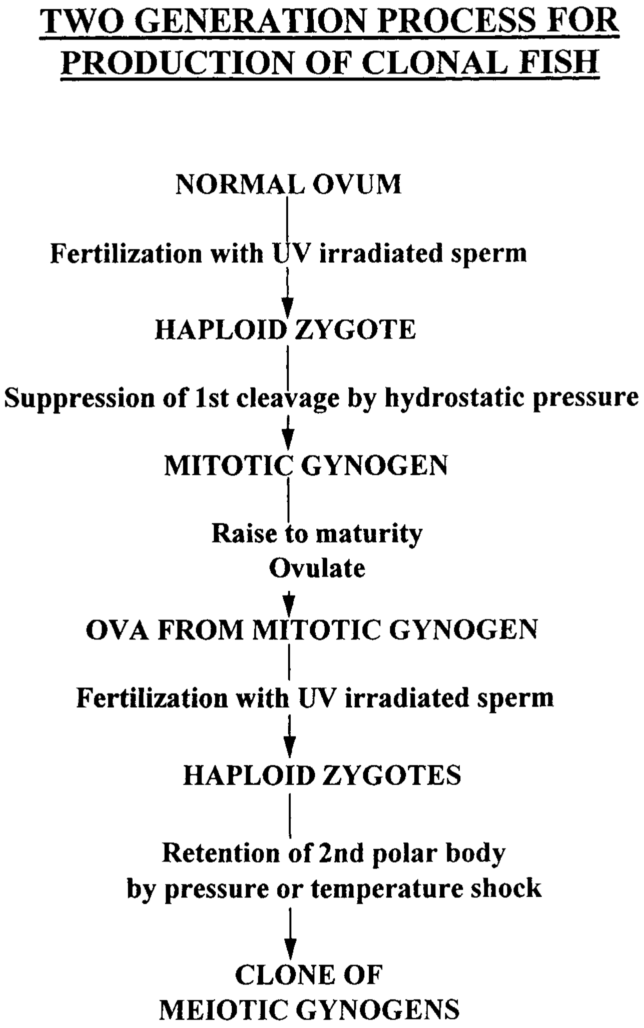
TWO GENERATION PROCESS FOR PRODUCTION OF CLONAL FISH
NORMAL OVUM
Fertilization with UV irradiated sperm
HAPLOID ZYGOTE
Suppression of 1st cleavage by hydrostatic pressure
MITOTIC GYNOGEN
Raise to maturity
Ovulate
OVA FROM MITOTIC GYNOGEN
Fertilization with UV irradiated sperm
HAPLOID ZYGOTES
Retention of 2nd polar body
by pressure or temperature shock
CLONE OF
MEIOTIC GYNOGENS

FIGURE 2. Two generation process for production of clonal salmonids. Modified from Taniguchi et al. (1988).

if the cloned salmonids are unsuitable for direct aquaculture use they would be valuable for gene mapping (Thorgaard 1992).

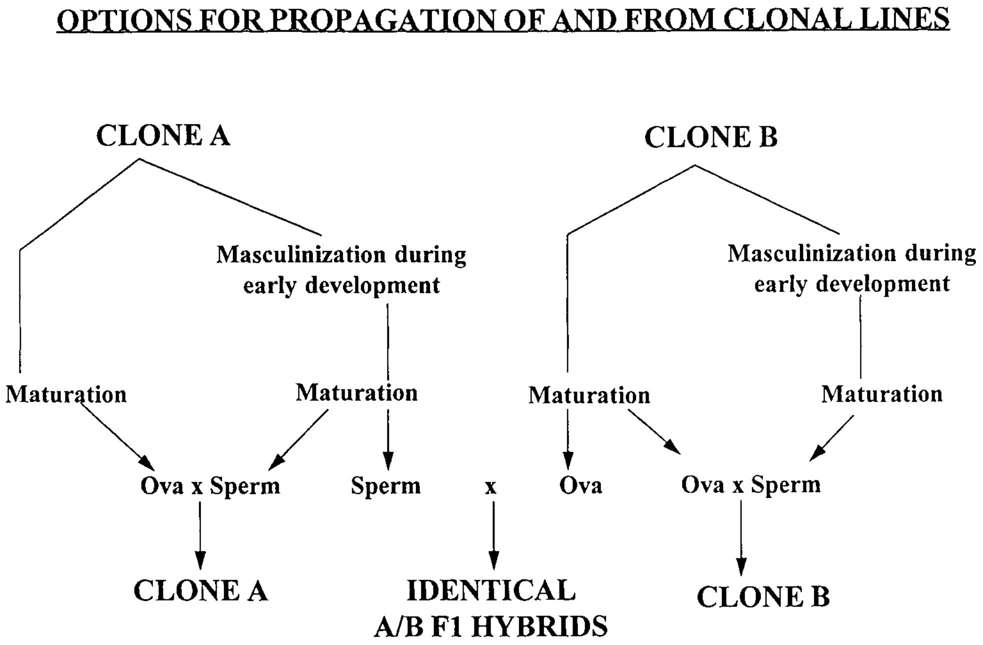
OPTIONS FOR PROPAGATION OF AND FROM CLONAL LINES
CLONE A
CLONE B
Masculinization during early development
Masculinization during early development
Maturation
Maturation
Maturation
Maturation
Ova x Sperm
Sperm
x
Ova
Ova x Sperm
CLONE A
IDENTICAL A/B F1 HYBRIDS
CLONE B

FIGURE 3. Options for propagation of and from clonal lines. Redrawn from Donaldson et al. (1995).

### *Summary*

Chromosome set manipulation is already being used on a production scale in aquaculture. Specifically triploid rainbow trout are being produced and marketed in North America and gynogenesis is being used as a tool for the production of monosex salmonid stocks. In the future we can expect increased utilization of triploidy as a means of sterilizing fish and thus eliminating reproductive interaction between escaped farmed fish and wild fish. We may also see androgenesis utilized to recover genetic information from cryopreserved sperm (gene banks) while androgenesis or gynogenesis may, after much further research, be used to produce identical cloned fish for both research and production purposes.

## CONTROLLED SEX DIFFERENTIATION

In many aquacultured species, one sex has better production characteristics than the other (e.g., rapid growth, delayed sexual maturation) or is more valuable in the marketplace, (e.g., species where the ovary [roe] has a high value). In other species or situations, it may be valuable to produce sterile fish and thus prevent sexual maturation, e.g., in salmonids where the development of secondary sexual characteristics results in decreased market value or where it is important to prevent reproductive interaction with wild fish. Several reviews of procedures for sex control in salmonids are available (Donaldson and Hunter 1983; Hunter and Donaldson 1983; Bye and Lincoln 1986; Piferrer and Donaldson 1988; Donaldson et al. 1993*a*, 1993*b*; Johnstone 1993; Piferrer and Donaldson 1993; Donaldson et al. 1995).

### *Monosex females*

*Direct Feminization.* Direct feminization refers to the process where salmonids are treated during early development in the embryo, alevin, or early feeding stage with an estrogen. When this procedure is carried out during the labile period for that particular species, all the fish develop as phenotypic females regardless of their genetic sex. The key variables which determine whether direct feminization will be successful include: (1) identification of the labile period, (2) selection of estrogen, (3) dose and duration of treatment, (4) method of estrogen administration. The labile period is the period during sex differentiation during which the developing fish is sensitive to the influence of exogenous sex hormones. In a recent study in chinook salmon, treatment by immersion in 400 $\mu$g/L 17$\alpha$ ethynylestradiol for 2 h just after hatching resulted in almost 100% phenotypic female offspring (Piferrer and Donaldson 1992). Similar treatments are effective in other salmonids. A caution regarding direct feminization for aquaculture is that the resultant fish are not suitable as broodstock since half are genetic males (i.e., XY) and would produce an increased proportion of males in the next generation. Furthermore, there is the issue of regulatory approval, although clearance of estrogen has been shown to be rapid (Piferrer and Donaldson 1994). For these reasons, indirect feminization (i.e., utilizing monosex female sperm) is preferred when feasible. Direct feminization has been used on a pilot production scale in coho (*Oncorhynchus kisutch*) and chinook salmon, but is not currently in commercial use in Canada.

*Indirect Feminization.* This procedure is preferred to direct feminization; however, in its original form it requires two generations to accomplish (Figure 4). The goal in female homogametic species, such as the salmonids, is to produce broodstock males that are genetically female. These phenotypic male, genotypic female broodstock fish produce only female sperm (XX) which, when used to fertilize normal eggs, produce all female offspring. The procedure involves in the first generation direct masculinization of mixed sex embryos, grow-out to sexual maturity, fertilization of normal eggs with the resultant sperm, progeny testing to determine which of the males was genetically female and a

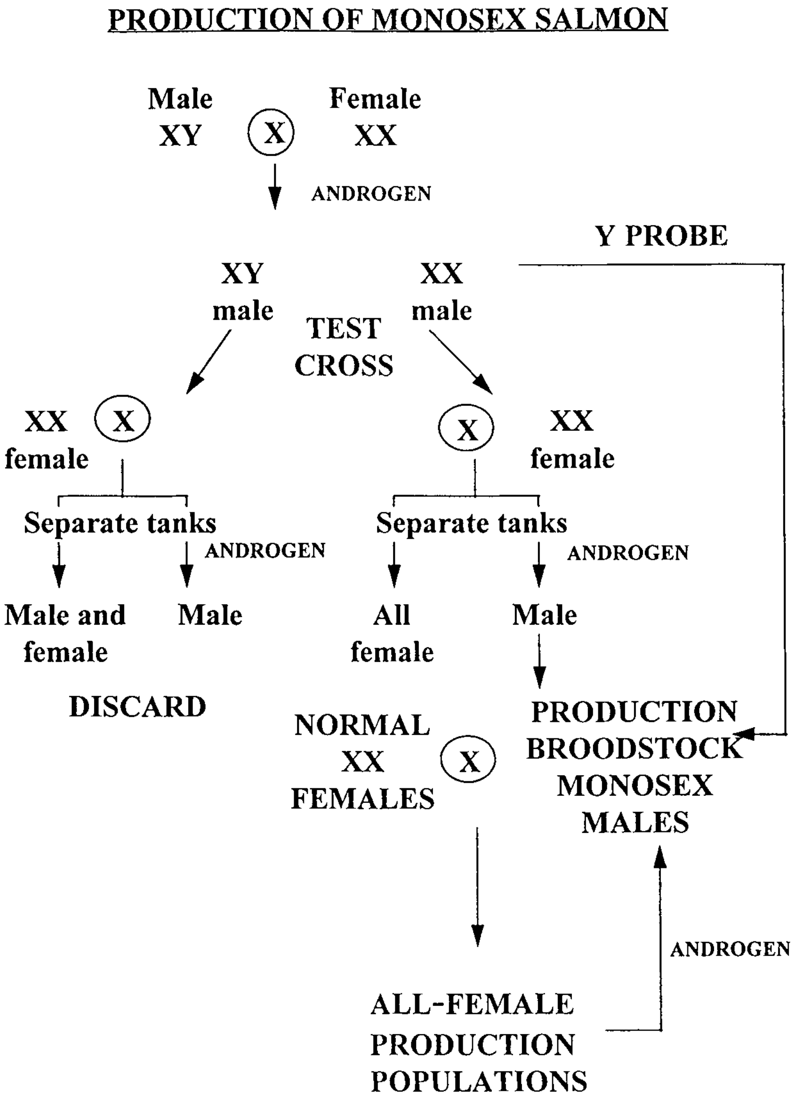

FIGURE 4. Protocol for producing monosex, all-female populations of salmon using conventional and Y-probe technologies. Modified from Devlin et al. (1994*b*).

second direct masculinization cycle for the embryos derived from the use of sperm from the phenotypic male, genotypic females. The grow-out of the fish from the second direct masculinization results in phenotypic male, genotypic female salmon which produce monosex female sperm (Donaldson 1986). Once monosex female embryos have been produced, it is simple to perpetuate the monosex female line by directly masculinizing a portion of the embryos to produce broodstock males which are genetically female (Donaldson and Benfey 1987) (Figure 5). This procedure has been used for the large scale commercial culture of monosex female chinook salmon in British Columbia since 1985 (Solar et al. 1987). The commercial culture of mixed sex chinook salmon is not economic as males mature on average one year earlier than females and large fish have a higher value per unit weight in the market (Solar and Donaldson 1991). The culture of monosex female or monosex female triploid rainbow trout is widespread in North America, Japan, and Europe. Monosex female triploid Atlantic salmon are being grown commercially in Tasmania (Jungallwalla 1991), and also have been grown on a trial basis in Scotland (Johnstone et al. 1991).

*Gynogenesis*. Gynogenesis offers promise as a means of producing monosex female sperm in a single generation (Figure 6). In homogametic female species, gynogenetic embryos are genetically female. These embryos can then be directly masculinized into phenotypic male gynogens that when mature produce monosex female sperm. This procedure has recently been used to produce monosex female rainbow trout (Feist et al. 1995), coho (Solar et al., unpubl. data), and Atlantic salmon (Galbreath et al. 1994; Solar et al., unpubl. data).

*Development and Use of DNA Sex Probes*. Until recently, no DNA sex probe was available for any species of fish. Unlike the situation in higher vertebrates, in many fish there is no visible difference in the karyotype of males and females and large differences in DNA sequence between sexes are not expected. A Y-specific probe (*see* below) capable of detecting the sexual genotype of chinook salmon regardless of sexual phenotype has been isolated (Devlin et al. 1991), and more recently, a Y-specific probe has been developed based on a salmon somatotropin pseudogene which can detect genetic sex in chinook, coho (Du et al.1993; Forbes et al. 1994), as well as pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon (Devlin et al., unpubl. data). Using the former probe, which has been licensed to industry, monosex female chinook salmon sperm has been produced in a single generation (Devlin et al. 1994*b*) (Figure 4). It is expected that the development of sex-specific DNA probes will greatly facilitate the monosex culture of other commercially important teleosts.

For species where sex-specific DNA probes are not yet available, XX males can be distinguished from XY males by: (1) test crosses (Hunter et al. 1983), (2) collection of sperm from masculinized individuals which retain ovarian tissue (Okada 1985), or (3) collection of sperm from orally masculinized individuals that fail to develop sperm ducts (Bye and Lincoln 1986).

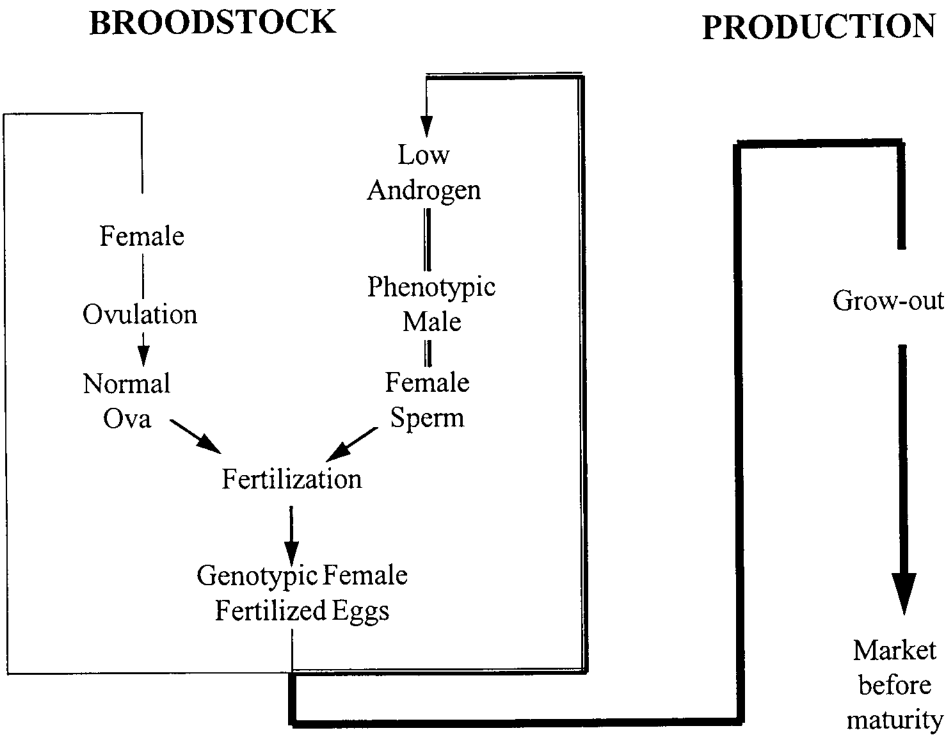

FIGURE 5. Production cycle for monosex female salmonids. Modified from Donaldson and Benfey (1987).

### *Monosex Males*

*Direct Masculinization.* This procedure involves the treatment of salmonids during early development with androgen to produce phenotypic males regardless of sexual phenotype. The treatment variables are similar to those for direct feminization (*see* earlier section). In salmonids, direct masculinization techniques have been worked out in considerable detail (Solar et al. 1984; Piferrer and Donaldson 1991; Piferrer et al. 1993; Johnstone and Maclachlan 1994) to facilitate the production of monosex female sperm.

*Indirect Masculinization.* There is as yet no commercial technique for the production of monosex male salmonid sperm. Indirect masculinization could be of interest for the rapid production of salmonids to market size providing that fish are harvested prior to the appearance of secondary sexual characteristics. In female

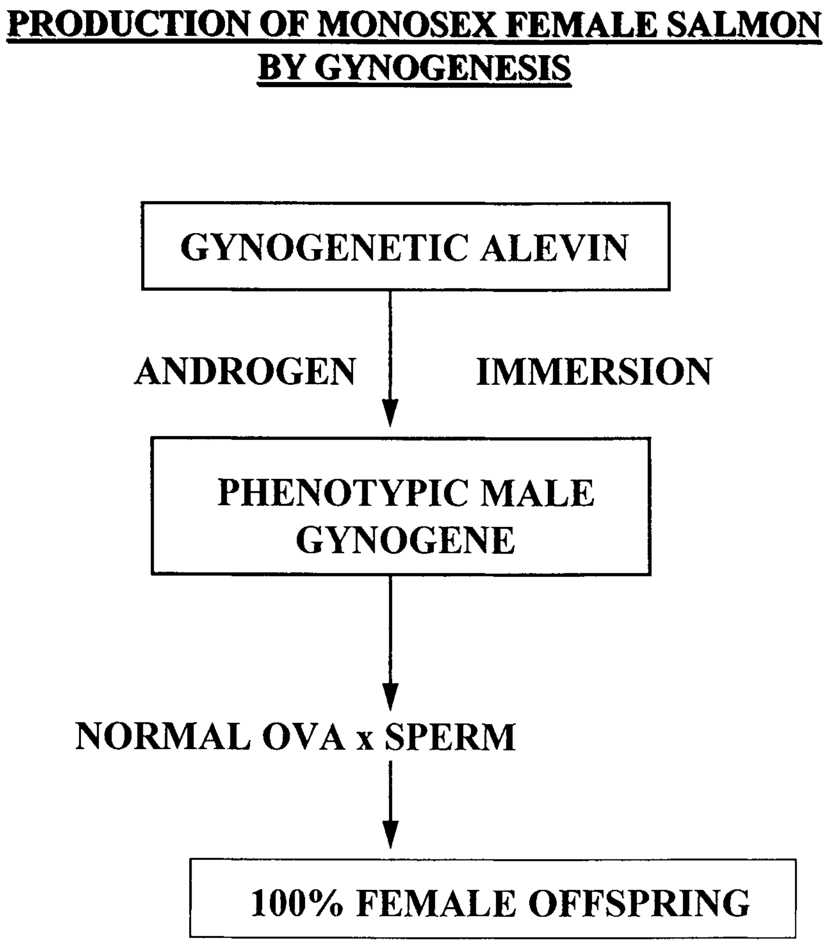

FIGURE 6. Production of monosex female sperm in a single generation by masculinization of gynogens. Modified from Donaldson et al. (1990).

homogametic (XX), male heterogametic (XY) species such as salmonids, monosex male sperm can only be produced by YY super-males, which have been shown to be viable in salmonids (Hunter and Donaldson 1983). YY super-males can be produced along with normal males by crossing sperm from a normal male with eggs from a genotypic male, phenotypic female produced by direct feminization. The super-males must then be separated from the normal males by progeny testing, by the strength of the signal detected by a Y-specific DNA probe, or by the use of a X-specific DNA sex probe yet to be developed.

*Androgenesis and Gynogenesis*. In male heterogametic species, such as the rainbow trout and other salmonids, YY super-males have been produced by androgenesis (Thorgaard et al. 1990). It remains to be seen whether this technique could be used to produce monosex male salmonids on a large scale. Male salmonids grow rapidly during the early stages of sexual development and could be an option for aquaculture. In salmonids the induction of gynogenesis in eggs from an XY phenotypic female, genotypic male fish would produce half super-males and half normal females. Meiotic gynogenesis may be technically easier to accomplish than androgenesis.

### *Sterilization*

*Review of Methods*. Sterilization has always played a major role in animal husbandry and in aquaculture. The use of sterile salmonids is expected to increase especially when sterilization can be integrated with growth acceleration technologies. In addition to the modification of performance or harvest quality by sterilization, there has been increasing interest recently in the sterilization of normal or genetically altered aquacultured fish as a means of preventing reproduction and thus genetic interaction between escaped aquacultured fish and wild fish (Devlin and Donaldson 1992; Donaldson et al. 1993*b*). There are about ten different techniques by which finfish may be sterilized (Donaldson et al. 1993); however, only a few of these are practical, reliable and acceptable from a regulatory point of view.

*Triploidy*. Currently for salmonids, the most favoured sterilization technique is the production of monosex female triploids (Johnstone et al. 1991). The production of monosex female triploids may be achieved (1) by use of monosex female sperm for fertilization before triploid induction, (2) by direct feminization after induction of triploidy, or (3) potentially by the use of monosex female diploid sperm from a phenotypic male, genotypic female tetraploid fish to fertilize normal eggs, thus producing allotriploids (Donaldson 1986) (Figure 7). Evidence is accumulating that monosex female triploid salmonids can eventually produce limited numbers of oocytes particularly when the fish are held for many years (Johnstone 1993; Benfey 1995; Donaldson et al., unpubl. data).

*Endocrine Techniques*. An alternative sterilization technique for salmonids is to expose fish during early development to high concentrations of androgen greater than is required to induce direct masculinization. In salmonids, exposure to androgen by immersion during the alevin stage is followed by dietary treatment for a period from first feeding (Donaldson et al. 1991). In rainbow trout dietary treatment alone is effective (Solar et al. 1984). Some success has recently been achieved with repeated or chronic immersion during the alevin stage, but 100% sterilization has not yet been achieved by androgen immersion in the absence of dietary treatment (Piferrer et al. 1994). Another potential method of inducing sterility in fish is by intervention in the hypothalamic-pituitary-gonadal axis. In recent studies, rainbow trout were injected with an extended

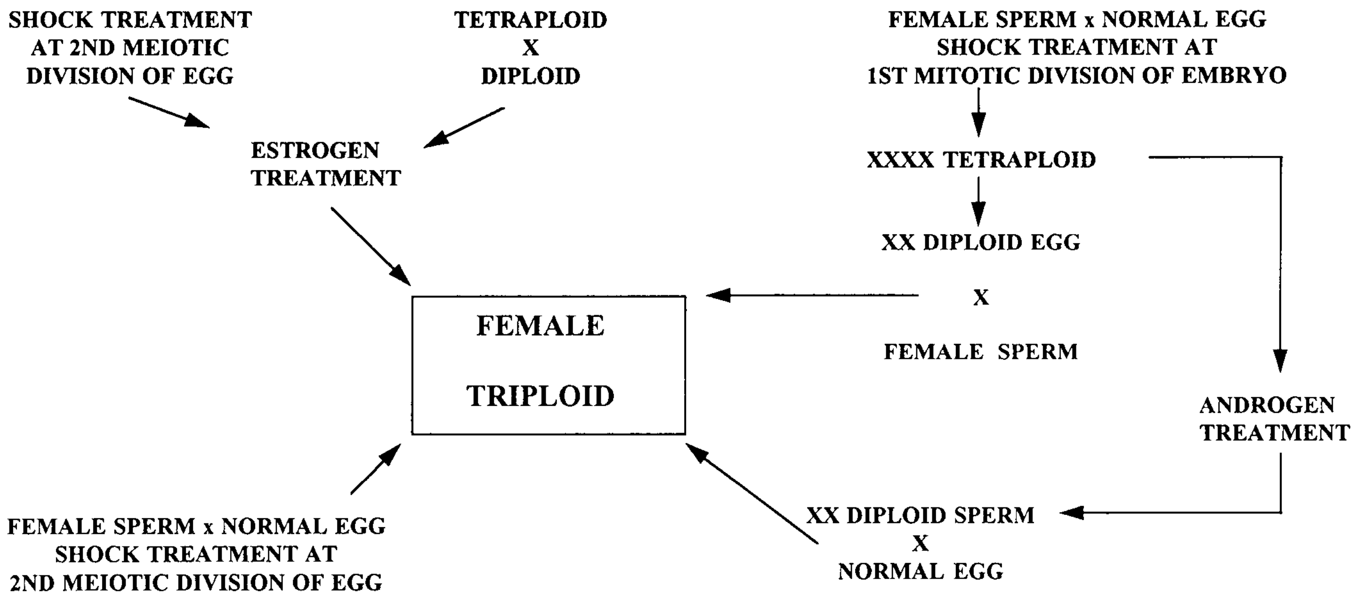
SHOCK TREATMENT AT 2ND MEIOTIC DIVISION OF EGG
TETRAPLOID X DIPLOID
FEMALE SPERM x NORMAL EGG SHOCK TREATMENT AT 1ST MITOTIC DIVISION OF EMBRYO
ESTROGEN TREATMENT
XXXX TETRAPLOID
XX DIPLOID EGG
X
FEMALE SPERM
FEMALE
TRIPLOID
ANDROGEN TREATMENT
FEMALE SPERM x NORMAL EGG SHOCK TREATMENT AT 2ND MEIOTIC DIVISION OF EGG
XX DIPLOID SPERM
X
NORMAL EGG

FIGURE 7. Alternative methods for the production of monosex female triploid salmonids. Redrawn from Donaldson (1986).

form of GnRH (mGnRH-Gly-Cys) linked to a carrier protein (purified protein derivative of tuberculin) (Riley and Secombes 1993). Anti-GnRH antibodies were developed after a single injection suggesting that it may be possible to inhibit sexual maturation in salmonids by immunoneutralization.

*Self-Sterilizing Transgenics*. While the production of monosex female triploid finfish is the most promising technique at the present time, the production of self-sterilizing transgenic fish may also be a possibility in the future (Devlin and Donaldson 1992; *see* later section).

*Role of Sterilization in the Culture of Genetically Altered Fish*. With rapid progress in the development of genetically-altered salmonids for aquaculture in many countries of the world, there is increasing public concern regarding the physical and biological containment of such stocks (Devlin and Donaldson 1992; Hallerman and Kapuscinski 1992). This is particularly true with regard to transgenic aquatic organisms. Several countries such as the U.K., Canada and the U.S. as well as the European Economic Community (EEC) are currently developing policies concerning the regulation of genetically altered aquatic organisms. It is expected that the use of sterile fish will be an important aspect of these regulations. The most critical issues in that regard are: (1) Can 100% sterilization be assured? and (2) What impact does sterilization itself have on the grow-out performance of fish?

### *Summary*

Sex control is proving to be of significant use in the optimization of salmonid culture systems. Prime examples from current world practice include the indirect production of monosex female chinook salmon and rainbow trout, and the production of sterile triploid female rainbow trout and Atlantic salmon. Key areas for research in sex control include: (1) the indirect production of monosex male populations; (2) the approval of specific androgens and estrogens for aquaculture use (in this regard, it may be possible to identify and use androgens that are not classified as restricted compounds); (3) the investigation of non-steroidal compounds, e.g., certain aromatase inhibitors, as masculinizing agents; (4) the improvement of methods for mitotic gynogenesis and tetraploidy; (5) the further refinement of methods for triploidy induction, including additional research on the production and utilization of diploid gametes; (6) studies on the performance of triploid organisms under production conditions; and (7) research on the production and performance of cloned salmonids to determine their suitability for culture.

## GROWTH ACCELERATION AND IMPROVED FOOD CONVERSION

The development of biotechnologies to improve growth rate and food conversion efficiency in aquaculture has long been a goal of aquaculture researchers (Donaldson et al. 1979). Despite the gradual improvement in these production characteristics through genetic selection programs and improved husbandry, the possibility of immediate improvement in the performance of existing strains remains attractive especially for slow-growing, cold water species with relatively long production cycles such as the salmonids. Environmental factors such as photoperiod and especially temperature play key roles in the determination of salmonid growth rates as does diet composition, feeding rate and feeding strategy (*see* Chapter 7). However, the focus here is on the pharmacological enhancement of growth and conversion efficiency. Recent reviews provide information on the environmental and physiological factors influencing growth in the rainbow trout (Sumpter 1992) and Pacific salmon (Weatherly and Gill 1995).

### *Endocrine Regulation of Fish Growth*

There are many endocrine factors — peptides, proteins, steroids and thyroid hormones — that are involved in the regulation of growth in fish. A simplified schematic diagram of the endocrine mechanisms involved is presented in (Figure 8). While steroid hormones and thyroid hormones are both capable of accelerating growth (Higgs et al. 1982) they are not current candidates for use in production settings due to regulatory or economic reasons, respectively. The role of insulin in fish growth processes has been recently reviewed (Sundby 1993).

### *Key Peptides and Proteins*

The production and release of growth hormone (somatotropin) from the pituitary gland in vertebrates is stimulated by growth hormone-releasing factor (somatocrinin) and inhibited by somatostatin. Growth hormone-releasing factor has been isolated and characterized from Pacific salmon (Parker and Sherwood 1990; Parker et al. 1993), but to date there are no reports of its use in *in vivo* growth studies. The role of somatostatin in fish growth regulation is still the subject of investigation (Diez et al. 1992). A recent study in chinook salmon suggests that it may be possible by administering a monoclonal antibody to somatostatin to block its effect and thus stimulate growth hormone release (Mayer et al. 1994). Growth hormone and related proteins are key candidates for the practical regulation of growth in fish. Growth hormone stimulates the synthesis and secretion of insulin-like growth factor(s) (IGF, somatomedin) in the liver and other tissues. IGF has been characterized from several salmonids, including coho salmon (Cao et al. 1989), rainbow trout (Shamblott and Chen 1992), and chinook salmon (Wallis and Devlin 1993). The amino acid sequence of the salmon IGF-1 is highly conserved having approximately 70% homology with mammalian IGF-1s. Treatment of chinook salmon

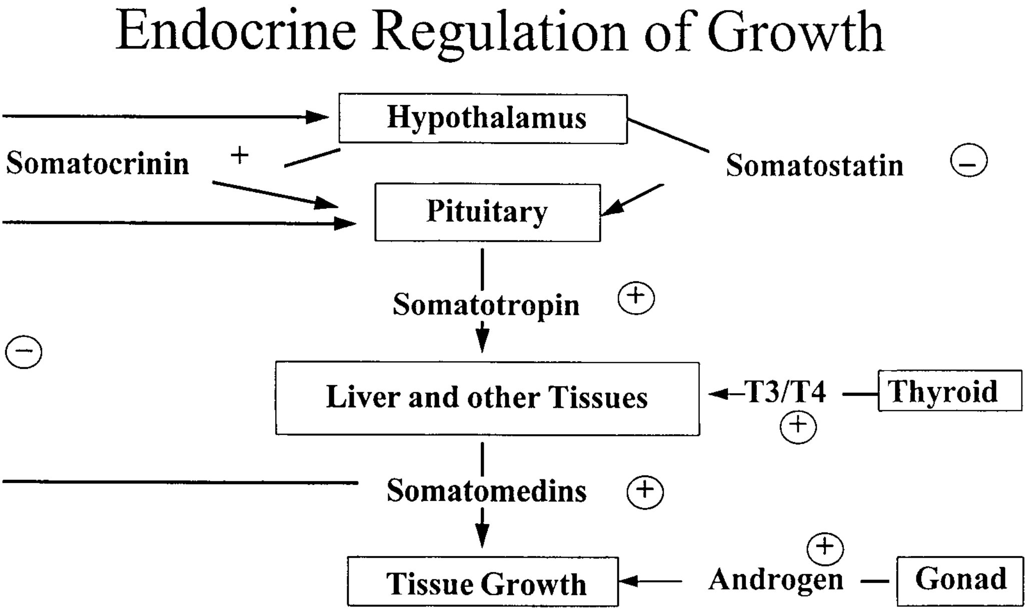

FIGURE 8. Key factors in the endocrine regulation of growth in teleosts.

with mammalian IGF-1 by osmotic pump resulted in dose-related mortality (Schulte, Down, Souza and Donaldson 1986, unpubl. data) and in brook trout (*Salvelinus fontinalis*) this mortality was preceded by a dramatic drop in plasma glucose and free amino acids (Skyrud et al. 1989). In coho salmon, slow-release administration of mammalian IGF-1 via osmotic pump resulted in increased growth (McCormick et al. 1992).

### *Somatotropin*

Of all the peptides, proteins, steroids and thyroid hormones tested to date for anabolic effects in fish, the approximately 20-kilo-Dalton (kD) proteins of the somatotropin family have been consistently the most effective. Endogenous growth hormone levels are less than is required for maximal growth in many teleosts, including the salmonids. In some other teleosts the availability of growth hormone is not limiting with regard to growth, and, in these species, the administration of exogenous growth hormone (GH) does not increase growth rate significantly (e.g., sea bream, *Sparus aurata*, Cavari et al. 1993; Ramos and Donaldson [unpubl. data in Donaldson and Down 1993]). Initial studies on growth acceleration in salmonids were conducted using natural mammalian or piscine GH (Higgs et al. 1976, 1977; Donaldson et al. 1979). When recombinant GHs became available they were also shown to be active in fish; recombinant avian and bovine GH were effective in increasing the growth rate of juvenile Pacific salmon (Gill et al. 1985). Since that time, there have been many studies with recombinant mammalian and piscine GHs in fish (reviewed by Down and Donaldson 1991; Sumpter, 1992; Le Bail et al. 1993; Donaldson and Down 1993; McLean and Donaldson 1993; Mayer and McLean 1995). The current focus of research and development on the use of somatotropin is to investigate the mechanism of action (Johnsson and Björnsson 1994) and to establish optimal treatment timing, dosage and formulation during the production cycle. Thus, in the production of salmon, GH could be used to: (1) accelerate the growth of presmolts during the freshwater stage and thus produce smolts in a shorter time or at a larger size, (2) treat presmolts just prior to smolting and seawater transfer so as to facilitate osmoregulatory adjustment during transfer and growth after transfer (Bolton et al. 1987; Boeuf et al. 1994; Sakamoto et al. 1994), and (3) accelerate growth and feed conversion efficiency during the grow-out period in seawater. Most studies to date have been conducted on presmolt or smolting salmon and there has been little study of growth acceleration during grow-out.

### *Placental Lactogen*

Placental lactogen is a member of the growth hormone-prolactin family, which has been isolated from the bovine and human placenta. Bovine placental lactogen (bPL) has recently been shown (Figure 9) to be very effective in accelerating both growth and smolting in salmon (Devlin et al. 1992, 1994*a*). Loss of parr marks in presmolt Pacific salmon was more rapid in bPL-treated fish than in bGH-treated fish and bPL-treated fish also grew faster than fish treated with the same dose of bGH. It is therefore difficult from this first study with bPL to determine whether the effect of bPL on smolting was solely due to its effect on growth. However, Shrimpton et al. (1995) have shown that bPL also can have rapid effects on osmoregulatory ability. Placental lactogen offers an alternative for accelerating growth in fish; however, further research would be required to obtain regulatory approval.

### *Modes of Administration*

Pituitary and placental hormones of the growth hormone-prolactin family can be administered to fish by immersion, orally or by injection. Immersion, while relatively effective as a means of vaccination, is probably not a very efficient means of growth hormone administration (Agellon et al. 1988; Schulte et al. 1989). Dietary administration shows considerable promise and may, with further research, become a practical means of administering GH and related proteins to fish (Moriyama et al. 1990; McLean et al. 1993*b*). In particular there is a need for further research on the co-administration of GH with compounds that either protect GH from the acid and enzymes of the digestive tract or enhance uptake from the intestine (McLean and Donaldson 1990). The most effective

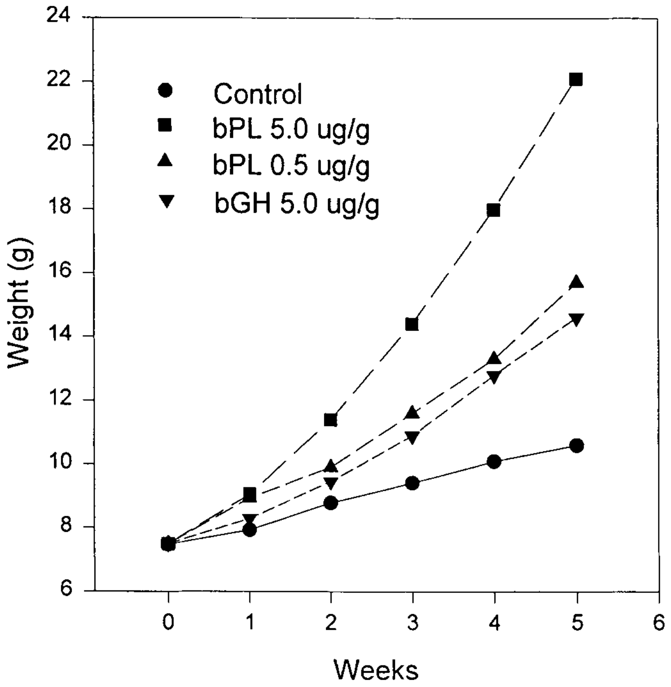

FIGURE 9. Growth stimulation of coho salmon using bovine growth hormone (bGH) and bovine placental lactogen (bPL). Redrawn from Devlin et al. (1994*a*).

means of administration developed to date involve injection or implantation of sustained release preparations or devices. In particular the injection of GH suspensions in oil has been effective (McLean et al. 1993*a*) as has the intraperitoneal implantation of miniosmotic pumps (Down et al. 1989*a*) and the implantation of polymer-coated GH (McLean et al. 1992). Use of this slow-release device resulted in growth acceleration for more than one year after initial treatment (McLean et al. 1994). Future research using recombinant DNA techniques may focus on the modification of the GH amino acid sequence to increase potency (Down et al. 1989*b*), thereby increasing the stability of GH in slow release preparations and the further development of homologous (i.e., salmonid) GH preparations for use in salmonid culture.

### *Regulatory Aspects*

The regulatory aspects of somatotropin use in fish will ultimately determine when such technologies are introduced. The recent decision (late 1993) to permit the use of bovine GH (bovine somatotropin, bST) with zero withdrawal period to increase milk production in dairy cows in the United States increases the likelihood of obtaining approval for the use of this and similar proteins in fish. It is probable that initial licensing approval would be sought for preparations which have already been characterized with regard to efficacy and human and environmental safety in domestic mammals. It could be argued that piscine somatotropins, especially if homologous, may be the most appropriate for use in fish. However, factors with regard to economy of scale during manufacture of the recombinant proteins and regulatory aspects may ultimately determine which growth hormones and which formulations are used first in aquaculture.

## MOLECULAR GENETIC DIAGNOSTICS

### *Introduction*

Diagnostic tests have become an important tool for the salmon farmer to improve husbandry techniques and include microbiological, immunological, chemical, and molecular genetic assays. The latter approach has only recently been implemented with the advent of simplified procedures for performing recombinant DNA research and the development of the polymerase chain reaction (PCR). DNA-based diagnostics have potential for application in a variety of areas including genetics and breeding, pathogen diagnosis, and evaluation of fish health (*see* Chapter 13).

### *Methodology*

Diagnostics using DNA technology employ methods that can be both extremely sensitive and very specific. They have the ability to identify sequences derived from specific locations within the fish genome (or transcripts derived therefrom), or from foreign DNA sequences derived from pathogens associated with the salmonid tissue. Detection methods for specific DNA sequences are based on the ability of complementary DNA molecules to hybridize together to form the native double-stranded DNA form, and when a "probe" sequence is labelled in some way (chemically or radioactively) or is allowed to initiate DNA synthesis, it can be used to identify the presence of like sequences in very complex mixtures of DNA. Three basic detection methods are commonly used: (1) blotting techniques (Southern or dot blotting), which transfers target DNA to a membrane for analysis; (2) the polymerase chain reaction (PCR), which is an amplification method that uses pairs of short DNA molecules (primers) to initiate DNA sequence replication between two defined points to generate DNA fragments of known size (that can be identified on electrophoretic gels); and (3) DNA-sequencing methods, which are now usually applied following PCR. Methods for most DNA procedures can be found in Sambrook et al. (1989).

### *Measurement of Genetic Variation in Cultured Salmonid Strains*

Genetic variation within populations provides opportunity to assess the potential plasticity and adaptiveness of a cultured strain to respond to artificial or natural selection pressures. Some genetic combinations (individuals) will prove superior in their ability to grow and survive in high ration and high density culture conditions and, in general, tend to lose the aggressive behaviour observed in wild strains, which must constantly compete for limited food resources. Thus, all cultured salmonid strains undergo selection due to rearing in net pens as the fish adapt to domesticated environments.

Genetic variation in salmonids exists in several different forms, and has been characterized using several different approaches. Most DNA-level variation that has been described is associated with anonymous loci, that is, those that are not associated with any known genetic trait or gene sequence. Nevertheless, monitoring this variation can provide an indication of what opportunity exists for change arising from selection, and what level of inbreeding has occurred in culture. Such variation is often found as structural differences in the genome (Wright 1993), usually in the form of variable numbers of tandem repeat sequences (VNTRs). VNTRs can be found in the form of microsatellites (2-6 base pair [bp] sequences which repeat up to several hundred bp) or minisatellites (consisting of larger repeats, consisting of a small conserved core sequence plus flanking regions which can extend for many kilobases in length). Minisatellites can be detected using Southern blotting or PCR, although the former is most often used either as a complex "DNA fingerprint" pattern, or by analyzing single chromosome regions at a time using single-locus probes that can reveal both alleles in a given individual (depending on the level of heterozygosity in the population). Microsatellites are usually identified on high-resolution agarose or acrylamide gels, and have the advantage that they avoid time-consuming blotting and hybridization methods. For salmonids, development and application of complex fingerprint probes (Lloyd et al. 1989; Taggart and Ferguson 1990a; Prodøhl et al. 1992; Bentzen et al. 1993; Stevens et al. 1993; Beacham et al. 1995; Heath et al. 1995), single-locus probes (Taggart and Ferguson 1990b; Bentzen and Wright 1993; Heath et al. 1993; Prodøhl et al. 1994; Taggart et al. 1995), and microsatellites (Estoup et al. 1993; Slettan et al. 1994; Sakamoto et al. 1994; Wright and Bentzen 1994) have been described.

DNA fingerprint information has the power to allow identification of siblings in mixed family salmonid populations (Stevens et al. 1993). This information may be used in breeding programs as a molecular tag that can allow efficient selection of desired family groups (for example, *see* "walk-back" selection; Doyle and Herbinger 1994). Heath et al. (1994) have also described a method using single-locus probes that can assess whether a phenotypic character that is polymorphic in a population has a genetic basis without actually undertaking a breeding study. Another culture-related application of DNA fingerprinting technology includes the identification of escaped farmed salmon in the wild (Bentzen et al. 1994; Withler et al. 1994) to allow estimation of environmental impacts.

Other types of variation that can be examined include Random Amplification of Polymorphic DNA (RAPD), mitochondrial genome variation (mtDNA), and sequence variation within structural genes of the nuclear genome. RAPD, being a PCR-based technique (Williams et al. 1990; Welsh and McClelland 1990), produces a fingerprint pattern of variable bands in salmonids (Dinesh et al. 1993) and is very fast, but it is more difficult to establish reproducible patterns. Analysis of genes in the mitochondrial (Thomas and Beckenbach 1989; McVeigh et al. 1991; Ferguson et al. 1993) and nuclear genomes is usually now conducted by PCR followed by direct sequencing. This method has the advantage that loci with defined function can be examined (i.e., growth hormone genes). Mitochondrial DNA has the further advantage of being able to define maternal lineages in the development of a strain.

One long-range goal of marker development is the construction of genetic linkage maps (*see* Postlethwaite et al. 1994) that define all chromosomal regions in salmon (*see* May and Johnson 1990). Although each salmon species has a different number of chromosomes, conservation of linkage arrangement is seen at the chromosome arm level for most species, and combining information from several species will be possible. The development of sufficient molecular genetic markers will ultimately allow their use in selective breeding programs using marker-assisted selection (Hallerman and Beckmann 1988). If anonymous polymorphic DNA markers are located sufficiently close to genes that influence quantitative characters, they can be used in breeding programs to select desirable genotypes independent of phenotypic expression of the trait (e.g., sex-limited characters, or traits which do not manifest themselves for several years).

### *Identification of Genetic Sex*

Protocols have been developed to produce strains with controlled sex differentiation, particularly all-female monosex strains. Using conventional breeding, coupled with androgen treatments to induce sex reversal in genetic females, the synthesis of monosex strains requires two generations and many single-family tanks to allow sorting of XX and XY males on the basis of test crossing. This procedure has been used to develop all-female strains of chinook salmon, coho salmon, and rainbow trout. To simplify this procedure and to accomplish it in one generation, DNA probes have been developed that allow identification of Y-chromosome-bearing individuals. Thus, by elimination of XY males, direct selection of sex-reversed, XX males can be made for use in crosses with regular XX females to generate all-female production populations (*see* Figure 4). A Y probe (OtY1) was initially developed for chinook salmon which required blotting technology (Devlin et al. 1991). However, most farm breeding protocols require rapid identification of XX males, and conversion of this test to a rapid PCR-based assay (Figure 10) was found to be desirable (Devlin et al. 1994). A Y-chromosomal DNA probe that is part of the growth hormone gene family has also been identified (Du et al. 1993; Forbes et al. 1994). This sequence, which is a non-functional GH pseudogene, is present in chinook, coho, chum, and pink salmon (Devlin et al., unpubl. data). To date,

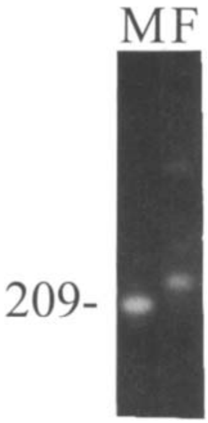

FIGURE 10. Agarose gel showing PCR-amplified products from male (M) and female (F) chinook salmon using OtY1 primers. Male-specific product is 209 base pairs in size.

no Y probes have been reported for Atlantic salmon or rainbow trout, but the latter species possesses cytogenetically distinguishable sex chromosomes (Thorgaard 1977).

### *Identification of Salmonid Pathogens and Fish Health*

A variety of pathogens (viruses, bacteria, parasites) infect cultured salmonids, which can result in very extensive losses (*see* Chapter 13). The epizootiology of these infections is not always known, and the onset and progress of the disease can be difficult to monitor in early stages when pathogen load is low and specific pathologies have not yet developed. DNA-based tests for salmonid pathogens have been developed for IHN and VHS viruses (Arakawa et al. 1990; Batts et al. 1993; Chiou et al. 1995), as well as for *Renibacterium salmoninarum* (Leon et al. 1994; Brown et al. 1994), *Aeromonas salmonicida* (Hennigan et al. 1989; Gustafson et al. 1992; Hiney et al. 1992; Nielsen et al. 1994), and *Vibrio* sp. ( Soerum et al. 1990; Wards et al. 1991) using either blotting or PCR technology. Molecular tests for microsporidian parasites (which can cause substantial losses to salmon farmers) are also currently being developed (Barlough et al. 1995; Docker et al. 1995). Testing usually involves isolating DNA from infected host tissue, and the sensitivity of the assays then depends on detecting the pathogen-specific sequences amongst all other host DNA. If infections are light, detection of pathogens can be difficult simply because insufficient template DNA can be analyzed in single molecular genetic assays. Thus, the sensitivity of molecular assays may not always be as high as conventional test methods where larger equivalents of tissue can be examined;

however, this problem can be alleviated to some degree by targeting repeat gene families (i.e., rDNA) in the diagnostic test. Since the specificity of these molecular tests is very high they can be very useful for determining strain type and, in general, molecular tests can be performed much more rapidly and cheaply than other diagnostic techniques.

Molecular genetic information is now available that can allow the physiological status of fish to be assessed. Heat-shock proteins are encoded by inducible genes involved in stress tolerance in most life forms, and several classes have been described. Also called stress proteins (SP), some are involved as molecular chaperons to detect and mark cellular proteins which have been damaged by abnormal conditions (e.g., anoxia, heat stress, disease challenges, chemical poisoning). In salmonids, SPs have been shown to respond to a variety of physiological stressors (Misra et al. 1989; G. Iwama, Univ. British Columbia, pers. comm.) and may be general indicators of the stress level in fish. Since both SP70 and SP30 have been cloned from salmon (Kothary et al. 1984; Hargis et al. 1995), the development of genetic assays for monitoring the expression of these genes is also possible. Similarly, genetic assays have been described for genes with other specific physiological roles. Metallothioneins are proteins involved in heavy metal sequestration and excretion, and the genes are induced on exposure to toxic metals (Misra et al. 1989). Two forms of metallothionein genes are expressed in salmonids (Zafarullah et al. 1990) and a general PCR assay has been described that can allow monitoring the expression of these genes from many fish species, including salmonids (Chan 1994). Recently, Campbell and Devlin (1995) described a PCR assay that can monitor the induction of the salmonid CYP1A1 gene that encodes a P450 enzyme involved in the excretion of xenobiotics (i.e., PCBs, PAHs, dioxins) derived from natural and industrial sources. Depending on the geographical location of aquaculture rearing facilities, these latter two methods could be used to determine directly whether exposure to environmental pollutants has occurred.

## TRANSGENIC TECHNOLOGIES

### *Introduction*

The development of recombinant DNA technology has resulted in improved knowledge of the structure and function of many genes from agricultural animals, including fish. A large number of genes have now been isolated and characterized from salmonids, and many of these are associated with commercially important traits. For example, genes involved directly in growth regulation include growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II), growth hormone-releasing factor (GRF), and Pit-I. From this emerging molecular genetic information, it has become possible to understand the expression of important genes and to modify their structure accordingly to alter regulatory properties. An overall strategy for producing transgenic fish is shown in Figure 11, which indicates the multigeneration protocol required to produce strains useful for commercial aquaculture.

Early gene transfer work in mice produced very dramatic effects, for example, doubling the size of mice containing mammalian GH (Palmiter et al. 1982, 1983) or GRF (Hammer et al. 1985) gene constructs. Indeed, it is this series of reports on growth acceleration technologies in higher vertebrates that motivated much of the existing work on transgenic fish during the past decade. The ability to grow fish more rapidly was immediately recognized as having great potential for improving the efficiency of production in aquaculture. The first gene transfer study in fish was carried out using goldfish (*Carassius auratus*) (Zhu et al. 1985), and was soon followed by reports in loach (*Misgurnus anguillacaudatus*) (Zhu et al. 1986), salmonids (Chourrout et al. 1986*b*; Maclean et al. 1987; Fletcher et al. 1988), and catfish (*Ictalurus punctatus*) (Dunham et al. 1987). Since that time, a large number of studies have been performed in more than 15 fish species (*see* reviews by Maclean and Penman 1990; Chen and Powers 1990; Houdebine and Chourrout 1991; Fletcher and Davies 1991; Hackett 1993). The generation of transgenic fish for aquacultural purposes has also been reported for loach (Enikolopov et al. 1989), carp (Chen et al. 1993), catfish (Dunham et al. 1992), and pike (*Esox lucius*) (Gross et al. 1992), as well as five salmonid species including rainbow and cutthroat trout (*Oncorhynchus clarki*), and Atlantic, coho and chinook salmon (Chourrout et al. 1986*b*; Maclean et al. 1987; Fletcher et al. 1988; Guyomard et al. 1989; Penman et al. 1990; Du et al. 1992; Devlin et al. 1994*c*, 1994*d*, 1995*a*). Initial studies with salmonids focused on gene transfer methodologies, but it was not until the early 1990s that modification of any phenotypic character was achieved in transgenic salmonids (Du et al. 1992; Devlin et al. 1994*c*, 1994*d*, 1995*a*, 1995*b*).

### *Approaches for Manipulating Phenotype*

Genetic improvement of terrestrial agricultural species has been under way for several thousand years, either through indirect selection or, more recently, in programs designed to specifically enhance particular performance traits. For salmonid aquaculture, strains have only recently been acquired from the wild and therefore have not undergone major genetic changes. Thus, significant variation that remains in cultured stocks allows improvement through genetic selection, with observed performance gains being on the order of 4-20% per generation (*see* Chapter 11). During selective breeding programs, the changes that are implemented are obscure. In almost all cases the genes involved in the improvements are undefined, although it may be possible to estimate the number of loci involved. Further, the physiological basis of the alterations are also usually unknown. In contrast, with genetic engineering, by the nature of the technology, the genes must be defined in considerable detail and the physiological basis of the process being influenced must be well understood. This provides the investigator with the knowledge and molecular tools to examine the effect of a transgene introduction, both for desired changes and for unanticipated effects arising from pleiotropic action of the transgene.

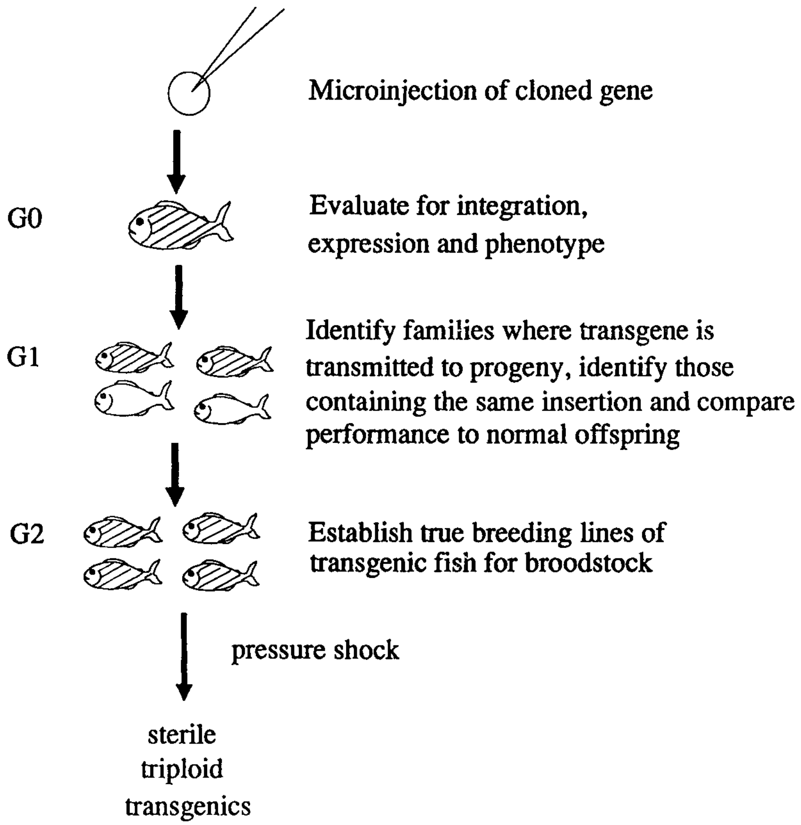
Microinjection of cloned gene
G0
Evaluate for integration, expression and phenotype
G1
Identify families where transgene is transmitted to progeny, identify those containing the same insertion and compare performance to normal offspring
G2
Establish true breeding lines of transgenic fish for broodstock
pressure shock
sterile triploid transgenics

FIGURE 11. Flow diagram showing the multigenerational procedure required to produce pure breeding lines of transgenic salmon.

Several molecular genetic approaches are available to change phenotypes in transgenic organisms depending on the physiological nature of the character being influenced (Table 1).

Transferring phenotypes between strains or species requires the molecular characterization of a genetic trait and the ability of the donor gene to function in a physiologically meaningful way in the host organism. For example, antifreeze genes isolated from the winter flounder (*Pleuronectes americanus*) have been transferred into Atlantic salmon (Fletcher et al. 1988) to produce freeze-resistant strains for culture in sub-zero water temperatures below sea ice in the North Western Atlantic Ocean. To date, successful transfer, integration and transmission of the antifreeze genes have been observed in transgenic Atlantic salmon (Fletcher et al. 1988; Shears et al. 1991) and it is

TABLE 1

Approaches to phenotypic modification in transgenic fish.

| Strategy | Examples |
|---|---|
| 1. Transfer phenotypes between strains/species | Disease resistance<br>Freeze/cold tolerance<br>Metabolic capabilities |
| 2. Augment phenotypes | Growth rate<br>Feed conversion efficiency<br>Disease resistance |
| 3. Remove phenotypes | Sexual maturation<br>Aggression |
| 4. Alter developmental appearance of traits | Smoltification<br>Sexual maturation |
| 5. Create novel phenotypes | Nutritional character of flesh<br>Behavioural modifications |

promising that expression can be detected, although thus far at an insufficient level to provide significant freeze resistance (Fletcher et al. 1992 ).

Augmenting phenotypes has to date been the most successful transgenic technology with salmonids. Most effort has been devoted to increasing growth rate by transferring growth hormone gene constructs into commercially important salmonid species (Chourrout et al. 1986*b*; Maclean et al. 1987; Guyomard et al. 1989; Rokkones et al. 1989; Penman et al. 1990, 1991; Du et al. 1992; Devlin et al. 1994*c*, 1994*d*, 1995*a*, 1995*b*). From our understanding of growth regulation in salmon it has been known for many years that their growth rate is below the maximum possible. In salmonids in particular, many studies have demonstrated that administration of proteins in the GH family can result in very dramatic increases in growth rate. Transgenic techniques have the potential to circumvent the major difficulty associated with GH therapy, that is the need for sustained or repeated delivery, by allowing the organism itself to produce elevated levels of GH (*see* below).

Removal of negative traits from cultured salmonid strains is desirable when such characteristics impact on the efficiency of production. Some traits have mixed benefits; for example, sexual maturation of salmonids has both negative and positive features for culture. Early maturation can, in some cases, occur before optimum market size and ultimately sexual maturation results in the deterioration of the product. However, the early stages of sexual maturation also can provide a growth enhancement effect via the elevated levels of gonadal steroids. Having control over the onset and development of sexual maturation is an important tool for the salmonid culturist. Using transgenic technologies it is theoretically possible to either block, delay or accelerate sexual maturation by (1) controlling the production of gonadotropins or their release factors (Alestrøm et al. 1992), (2) controlling the expression of gonadal steroid-producing enzymes (Tanaka et al. 1992), or (3) interfering with the production of an essential component required for gonadal development. In some cases, it may be possible to limit these effects to either males or females by using genes that are exclusively expressed and required in one sex.

The creation of novel characteristics is also possible using transgenesis. For example, modification of the nutritional composition of the flesh is possible using gene constructs to control fat content and type (i.e., omega 3 and 6 fatty-acid ratio), or by adding components to the flesh with health benefits or commercial value which are not normally found in salmonids.

### *Genetic Elements Available for Construct Synthesis*

The ability to influence traits by transgenesis depends on the availability of suitable cloned genes that can be used to design new constructs with novel regulatory properties. The source of genetic material for transgenic experiments is important for two reasons: (1) although genes isolated from non-salmonid species may function to some degree, it is very likely that homologous genes (or as close as is possible) will be best recognized and respond to the host salmonid gene regulatory machinery and will be efficiently expressed, and (2) from the perspective of the potential consumer, it is desirable, where possible, to transfer DNA into the host organism that it already possesses.

Since the mid-1980s, a large number of genes have been isolated from salmonid species that are available for the manipulation of phenotpye in transgenics. A current survey of Genbank (the repository for DNA sequences) shows over 40 different classes of salmonid structural genes coding for physiologically important proteins. Examples are shown in Table 2, with potential approaches for their utilization.

To alter the expression of a gene it is necessary to have the DNA in cloned or PCR-amplified form, and usually knowledge of the complete nucleotide sequence is desirable. In general, genes are composed of two main structural regions, the coding sequence and the regulatory regions. A major component of the regulatory sequence is

TABLE 2

Cloned salmonid genes and examples of potential uses in transgenic fish.

| Physiolgical process | Cloned genes | Potential control |
|---|---|---|
| Reproduction | Aromatase | Sex reverse males |
| | Estrogen receptor | Sterilization/maturation |
| | Gonadotropin-releasing hormone | Induce/ablait maturation |
| | Gonadotropin subunits | Induce/ablait maturation |
| | Protamine | Sterilize males |
| | Steroid 17-$\alpha$ mono-oxygenase | Steroidogenesis |
| | Vitellogenin | Sterilize females |
| Growth | Growth hormone I and II | Growth acceleration<br>Induce smolting<br>Improve feed conversion efficiency |
| | Insulin-like growth factors | Growth acceleration |
| | Pit-I | Growth control |
| Disease | Complement C3 | Enhance nonspecific immunity |
| | Complement C9 | Enhance nonspecific immunity |
| | Fibrinogen | Wound healing |
| | Ig Recombination activating protein | Immunoglobulin type/diversity |
| | Immunoglobulin light chain | Germline immunity |
| | Immunoglobulin heavy chain | Germline immunity |
| | Lysozyme | Enhance nonspecific immunity |
| | Major histocompatability complex | Strain-specific resistance |
| | Mx protein | Viral resistance |
| | Tumour suppressing protein | Control proliferative infections |
| | Transferrin | Bacterial resistance |
| | VHS g gene | Viral resistance |

TABLE 2 cont'd.

| Physiolgical process | Cloned genes | Potential control |
|---|---|---|
| Other physiology | Aldolase | Carbohydrate metabolism |
| | Creatine kinase | Swimming performance |
| | CYP1A1 | Xenobiotic tolerance |
| | Glucagon | Carbohydrate metabolism |
| | Heat shock proteins 30 and 70 | Stress control |
| | Haemoglobin | Hypoxia resistance |
| | Insulin | Carbohydrate metabolism |
| | Isotocin | Osmoregulation |
| | Metallothionein A and B | Heavy metal resistance |
| | MyoD | Muscle content |
| | Myosin | Flesh quality |
| | Na-H exchange protein | Osmoregulation |
| | Preopiomelanocortin | Control stress |
| | Prolactin | Control osmoregulation |
| | Serum albumin | Osmolarity control |
| | Tropomyosin | Flesh quality |
| | Vasotocin | Osmoregulation |

Note: Genbank search conducted using keywords 'Oncorhynchus' OR 'Salmo' AND 'Osteichthyes' via retrieve @ ncbi.nlm.nih.gov. References can be found therein.

the promoter, located immediately upstream of the coding region, which controls the level of gene transcription and confers tissue-specific and temporal control over expression. Different genes possess distinct promoter regions with unique regulatory properties, and these regulatory regions can be exchanged between coding regions to produce gene constructs with novel regulatory properties. A variety of promoter sequences have been used in transgenic fish studies, including sequences from viral, mammalian, insect, and piscine sources, and many of these have been tested in salmonids in tissue culture or transgenic individuals (*see* Fletcher and Davies 1991; Devlin et al. 1994*d*).

### *Methods of DNA Introduction into Salmonid Zygotes*

Several methods have been explored for introducing gene constructs into fish cells or embryos. The classical method of microinjection, which has proven effective in many systems (mice, frogs, insects), has also been successful as a gene transfer system in fish.

Microinjection uses a fine glass needle (approximately 5-$\mu$m tip diameter) controlled by a micromanipulator to physically deliver the gene construct into eggs or early embryos. Although a considerable range of DNA concentrations has been used, between 10 and 100 ng/$\mu$l appears to be effective, corresponding to approximately $10^6$ copies of the gene construct injected (depending on the size of the DNA molecule used).

In the case of salmonid eggs, fertilization is usually conducted in fresh water and is accompanied by activation of the egg and initiation of embryonic development. Egg activation is accompanied by a hardening of the external chorion layer of the egg such that within a few minutes, the introduction of fine microinjection needles becomes more difficult. Fortunately, from the work of Ginsburg (1963), it has been known for many years that the processes of fertilization and chorion hardening can be separated. Since chorion hardening is initiated by exposure to hypotonic solution (*see* Chapter 5), eggs fertilized in an artificial ovarian fluid solution remain soft and can be penetrated by an injection needle. Some researchers working with rainbow trout have injected through the hardened chorion after drilling a small entrance hole through which the microinjection needle could be inserted (Chourrout et al. 1986*b*).

DNA is usually delivered into fertilized salmon eggs in a volume of 1-2 nl. Injection technique requires introduction of DNA below the perivitelline space, through the vitelline membrane, and into the egg cytoplasm (*see* Figure 10 in Chapter 2). Since the location of the pronuclei cannot be visually discerned, injection is usually targetted to the central blastodisc region. Researchers working with Atlantic salmon have delivered DNA through the micropile, the canal through which the sperm migrates to the egg during fertilization (Shears et al. 1992).

The reported success of gene retention in various salmonid species ranges from a few to over 50% (Chourrout et al. 1986*b*; Fletcher et al. 1988; Guyomard et al. 1989; Penman et al. 1990, 1991; Du et al. 1992; Devlin et al. 1995*a*). However, the same methods have not been used in all studies to identify the introduced genes, and analysis has been performed at different developmental stages. Since virtually all injected animals initially contain the gene construct and much of the injected DNA is lost as development proceeds, repeatable determinations of gene retention frequency (i.e., the same individual will consistently be positive) are only observed after a significant period (at least six months). In their experiments, Devlin et al. (1994*d*, 1995*a*) found stable gene retention frequencies ranging up to 13.7%, depending on the gene construct and species employed.

Microinjection is a slow and technically demanding procedure, which limits its application on a large scale. To overcome some of these limitations, other methods have been explored to deliver gene constructs to large numbers of individuals or gametes simultaneously. Electroporation employs a high field-strength, short-duration electrical pulse that renders the plasma membrane of cells permeable and can allow passage of DNA from outside of the cell. In salmonids, electroporation of sperm has been investigated as a gene transfer method (Symonds et al. 1994*a*, 1994*b*), and evidence of

DNA-sperm association has been obtained. Other methods of mass gene transfer under exploration in aquatic organisms include biolistic introduction which uses a "gene gun" to introduce DNA coated on small particles at high velocity into cells (Gendreau et al. 1995), and the use of liposomes (lipid bilayers containing DNA that are capable of fusing with plasma membranes), which have been successfully used for transgenesis in chickens (Squires and Drake 1993). Recently, retroviral vectors with a broad host range have been used successfully to generate transgenic zebrafish (Lin et al. 1994); however, such vectors may have limited application for agricultural species used for human consumption. An alternative approach to germ-line transformation is also being explored in fish by using direct injection of gene constructs into muscle tissue to allow expression to occur from somatic tissues (Rahman and Maclean 1992). In this case the concern over propagation of the transgene by escaped fish is eliminated, although each individual production fish would require treatment. In salmonids, no method other than microinjection has yet been shown to produce germ-line transformed transgenic individuals.

Injected DNA inserts into the chromosomes of vertebrates via a presently undefined mechanism. In fish, as in the transgenic mouse system, DNA can insert in catenated structures (Dunham et al. 1987; Guyomard et al. 1989; Devlin et al. 1995*a*) in all three conformations (head to head, tail to tail, and head to tail). Since the number of copies that insert into the chromosomes ranges from one to several hundred, with multiple sites of insertion potentially occurring in each genome, the genetic structure of founder transgenic fish can be quite complex.

Stable transmission of introduced gene constructs between generations can only occur if the DNA-insertion event occurred in an embryonic cell destined to become part of the germ-line lineage. In salmonids, the frequency of transmission from $G_0$ parents ranges from 0 to 64% (Guyomard et al. 1989; Penman et al. 1991; Shears et al. 1991; Devlin et al. 1995*b*), but is usually less than that expected for Mendelian transmission (50% for a single locus insertion, 75% for two loci, etc.). Frequencies lower than 50% suggest that a mosaic distribution of the transgene exists in founder transgenic fish due to the integration of the injected DNA into a host chromosome after the first cell division has already occurred. This results in germline tissue that is a mixture of transgenic and nontransgenic cells, and the ratio of the two will influence the frequency of transmission. In subsequent generations ($F_2$) where the transgene has been acquired in the $F_1$ individuals by transmission in sperm or eggs, the transmission frequency follows Mendelian rules (Shears et al. 1991).

### *Growth Enhancement in Transgenic Salmon*

As mentioned earlier, considerable effort has been devoted to transgenic technologies that could enhance growth rate, final size, and feed conversion efficiencies in fish. The limited production of GH in salmonids is undoubtedly appropriate for optimum survival in nature based on feed availability and life history requirements. This regulation is achieved through a complex regulatory process involving stimulation of GH gene expression (via GRF), GH clearance from serum (due to regulated numbers of GH receptors on target tissues), and by homeostatic feedback regulation acting at the level of the pituitary gland.

GH production is restricted to the pituitary gland, and may be sensitive to serum GH levels such that elevated levels can result in down-regulation of GH secretion. To produce elevated levels of GH to increase growth for aquacultural situations, both the tissue-specific expression and the feedback control system should be supplemented with a construct containing a promoter which can confer high levels of GH synthesis. For the over-expression of GH genes in salmonids, two non-fish constructs (SV40 and mouse metallothionein promoters driving the expression of rat or human GH genes), one "all-fish" fish construct (opAFPGHc: an ocean pout, *Macrozoarces americanus*, antifreeze protein promoter driving the expression of a chinook salmon GH1 cDNA; Du et al. 1992; Devlin et al 1995*a*), and one "all-salmon" construct (pOnMTGH1: a sockeye salmon, *Oncorhynchus nerka*, metallothionein promoter fused to the sockeye salmon GH1 gene; Devlin et al. 1994*d*) have been reported. As some studies have not allowed microinjected fish to develop to a point where growth could be monitored, it is difficult to make meaningful comparisons among experiments. However, in those studies where long-term retention of introduced DNA has been demonstrated (Guyomard et al. 1989; Penman et al. 1991; Du et al. 1992; Devlin et al. 1994*d*, 1995*a*), it appears that those constructs composed of fish DNA have resulted in stimulation of growth, whereas gene constructs derived from viral or mammalian sequences have not. The overexpression of GH results in increases in growth rate such that after about one year of development, transgenic Atlantic salmon are 3-5-fold larger and transgenic Pacific salmonids (chinook and coho salmon, and rainbow and cutthroat trout) average 11-fold heavier than their non-transgenic siblings. Although this dramatic effect on growth rate (*see* Figure 12) can produce fish as large as 37-fold heavier than controls at one year of age (Devlin et al. 1994*d*), the fish do not ultimately reach giant size; growth-accelerated transgenic Pacific salmon mature at approximately normal weight, but they reach this size of maturity much sooner than their nontransgenic counterparts. Thus, the physiological alterations appear to be largely acting on growth rate, and the magnitude of the difference in size between transgenic and non-transgenic individuals changes throughout development.

Growth hormone treatment has also been shown to accelerate the onset of the parr-smolt transformation (Sweeting et al. 1985; Bolton et al. 1987; Shrimpton et al. 1995), an effect that could allow early introduction of production fish into sea-pen growout conditions. In the cases where growth stimulation has been observed in transgenic salmon, precocious induction of the parr-smolt transformation has been observed (Du et al. 1992; Devlin et al. 1994*d*; 1995*a*, 1995*b*). The opAFPGHc and pOnMTGH1 fish gene constructs are presumably driving the expression of hyperphysiological amounts of GH from extrapituitary tissues. Interestingly, while elevation of serum GH levels can be observed in Pacific salmon with both the

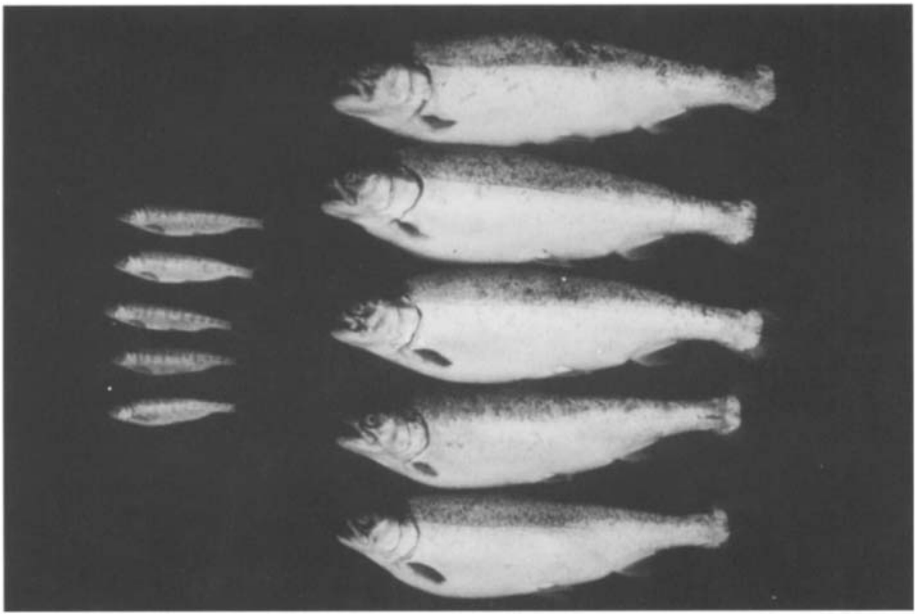

FIGURE 12. Growth-enhanced transgenic coho salmon (right) shown beside their nontransgenic siblings (left). Note loss of parr marks in transgenic individuals.

pOnMTGH1 and opAFPGHc constructs (Devlin et al. 1994*d*; unpubl. data), elevated levels of GH have not been observed in Atlantic salmon with the latter construct (Du et al. 1992). Using these constructs in Pacific and Atlantic salmon, the very largest fish can display skeletal abnormalities (excessive cartilage growth in the head, jaw, and operculum) analagous to acromegaly syndrome seen in higher vertebrates, which arises from pathological over-expression of GH (Devlin et al. 1995*a*, 1995*b*). Thus, for selection of appropriate broodstock, it will be desirable to breed those individuals that exhibit accelerated growth but also display a normal phenotype. Before growth-enhanced transgenic salmonids can be considered for application in aquaculture, a great deal of investigation is required to examine aspects such as feed conversion efficiencies, flesh nutritional quality and palatability, disease and stress resistance, and reproductive capabilities.

Transmission of the inserted GH gene constructs to $F_1$ progeny has been demonstrated for both opAFPGHc and pOnMTGH1, and in both cases the accelerated growth phenotype is transmitted as well. Although inactivation of foreign DNA has been reported in transgenic mice and zebrafish (McGowan et al. 1989; Stuart et al. 1990), inactivation of inserts for gene constructs composed of fish DNA may not be a problem in salmonids (DNA sequences that have evolved in a fish genome may be less susceptible to chemical modification and inactivation by fish nuclear enzymes). The long-term retention of transgenes in $G_0$ individuals, as well as the transmission to $F_1$ progeny, are consistent with but do not demonstrate that the foreign DNA has integrated into host chromosomes. However, Southern blot data (Maclean et al. 1987; Fletcher et al. 1988; Guyomard et al. 1989; Penman et al. 1991; Devlin et al. 1995*a*) and, in one case, *in situ* hybridization (Tewari et al. 1992) data have provided strong evidence that, in most cases, transgenes in salmonids are contiguous with host DNA.

### *Development and Application of Broodstock*

Figure 11 shows the multi-generation protocol required to synthesize transgenic fish strains homozygous for a transgenic locus that would breed true for the associated phenotypic trait. Each founder transgenic fish is genetically unique, and each should be bred separately to allow development of strains with defined genetic structures and characteristic phenotypic effects. Using conventional breeding methods, three generations are required to develop true-breeding lines, and for salmonids this can range from 6 to 12 years depending on the species and trait being modified (growth-enhanced fish mature sooner than normal and can shorten this time). Genetically pure lines of transgenic fish (homozygous with respect to the transgene) are desirable as either (1) they allow use directly in production or (2) they can be used as broodstock to cross with other strains to produce heterozygous fish for production populations. While both approaches ensure the production of 100% transgenic individuals for production, outcrossing homozygous transgenics to established non-transgenic broodstock has the advantage of allowing the introduction of novel genetic diversity into production populations for improved disease resistance or other characteristics. If single families are initially used in strain development, the production of homozygous lines of transgenic fish from individual founders unfortunately produces stocks inbred for other genetic loci as well; this could be avoided to some degree if it is possible to raise many families per founder transgenic parent. Crossing founder transgenic fish among themselves could also be used to increase the number of transgenic loci present within a strain, but the production of pure breeding, homozygous lines becomes progressively more difficult as additional loci are added. To differentiate heterozygotes and homozygotes, it is necessary to use either gene dosage analysis (on Southern blots) or to clone and characterize the insert site to allow wild type and insert-containing chromosomes to be distinguished.

The number of generations required to produce pure lines can be reduced in salmonids by incorporating gynogenesis or androgenesis in the breeding program (*see* earlier section). These procedures can produce individuals homozygous for transgenic loci in two generations rather than three, although for early gynogenesis, heterozygous transgenic individuals may also be produced if the insertion site is in a distal region of the chromosome. In the case of gynogenesis, genetically all-female populations will result and thus sex-reversal procedures are also required to produce transgenic males to perpetuate the pure line.

It is desirable to evaluate the phenotypic trait for production characteristics in $F_1$ or $F_2$ progeny rather than in founder $G_0$ transgenic fish. Due to the mosaic distribution of the transgene in $G_0$ fish, not all cells in founder fish will be transgenic. Thus, for endocrine factors, expression of the modified trait will be less than in subsequent generations and for cell-autonomous traits, not all cells will express the characteristic (e.g., for transgenics containing sterilization constructs, complete infertility would not be expected in founder fish). Similarly, it is important to decide whether heterozygous or homozygous fish will be cultured, as the difference in gene dosage may have important effects on the phenotype.

### *Potential Environmental Impacts, Containment Methods, and Regulatory Requirements*

Salmon cultured in sea-pen cages can escape from their physical containment due to damage from weather or predators, or they can be accidentally released during handling and processing of the fish at several points during the production cycle. The potential impact that transgenic fish may have on ecological systems is difficult to predict and is highly dependent on a number of factors relating to the fitness of the transgenic individuals relative to wild populations. While the fitness of transgenic fish will depend on which phenotypic trait is being modified, and the degree of deviation from wild type, in practice it is very difficult to estimate *a priori* the relative fitness of a genetically distinct strain when it cannot be tested in the same environment (i.e., testing transgenic salmon in the wild would require their release). Some insight may be gained by examining important parameters (e.g., fertility, fecundity, swimming ability, disease resistance, foraging ability) of transgenic organisms in laboratory or mesocosm environments, but it is unlikely that these would prove to be completely reliable predictors of competitive ability in the wild. Even when differences in traits between transgenic and wild individuals appear very large, many genotypic alterations produce pleiotropic effects that may not be detected unless specifically tested for. Further, genotypic changes (transgenic or otherwise) produce phenotypic effects that are dependent on the environment that the fish is grown in. In the case of growth-enhanced transgenic salmon, significant increases in appetite and aggressiveness in foraging are observed in aquaria, and it is conceivable that such behavioural changes may allow transgenic fish to out-compete their wild counterparts (conspecifics or otherwise) in limited food resource situations or for limited space in breeding grounds. However, it is also possible that such fish with enhanced metabolic requirements may not be able to find sufficient resources fast enough in the wild and would experience nutrient limitations that would be detrimental to their overall performance. Similarly, transgenic fish may be devoting too much energy to growth processes rather than other aspects of performance, for example, immune function, and may experience reductions in viability. Although GH levels in transgenic fish may be optimized for performance in culture situations, it is very likely that wild fish have evolved optimum levels of GH for survival in nature. Again, this unfortunately does not mean that short-term impacts of transgenic fish on wild populations could not occur, particularly if large numbers of cultured fish were introduced into the wild. The ecological risks associated with transgenic fish have been reviewed (Hallerman and Kapuscinski 1992).

The regulatory requirements for the implementation of transgenic fish will involve safety issues regarding the nutritional and health aspects of the product, and will also require meeting guidelines (now being developed) to ensure protection of wild populations of fish (Hallerman and Kapuscinski 1992) and to ensure that only food products meeting established safety guidelines reach the consumer. Issues that undoubtedly will require examination include: (1) the nutritional quality of the fish (Does it deviate from wild or cultured products?); (2) whether any new products are present that are not normally observed in the host organism (e.g., novel proteins) or whether levels of naturally occurring compounds are affected by the transgenic alteration; (3) the source of the DNA used to make the gene construct (i.e., DNA from salmon, other fish, or more distant sources), although this may be more important for the issue of consumer acceptance of transgenic products; and (4) the stability of the gene inserts and method of gene introduction (e.g., microinjection, viral vectors). Performance standards designed to limit environmental impacts have been recently developed in the United States (USDA 1995*a*, 1995*b*), and a comprehensive evaluation of transgenic fish and the potential for their implementation has been undertaken in the U.K. (Woodwark et al. 1994). The voluntary guidelines in the U.S. are based on a risk assessment approach where investigators can assess transgenic fish for their ability to cause harm in a particular environment and appropriate measures can be taken. In Canada, guidelines require that culture of native transgenic fish be conducted in secure laboratory facilities, and due to the difficulties of determining the potential impact of escaped salmonids in oceanic, coastal, estuarine and freshwater environments, the culture of transgenic organisms in these more natural environments could only be conducted with completely sterilized individuals (Canada DFO 1994).

To avoid long-term environmental impacts, containment of transgenic salmon in culture situations is required and involves both physical and biological strategies (Devlin and Donaldson 1992). Obviously, sufficient physical containment is required to anticipate any normal net-pen damage that might be incurred from natural causes. Equally important is effective training of personnel that handle transgenic fish; the focus of culture must ensure that transgenic individuals are not accidentally released. However, despite such precautions it is a certainty that some transgenic salmonids cultured in sea pens would eventually escape and as such, it is necessary to consider additional measures of containment to prevent reproductive interaction with wild fish. Although a variety of methods currently exist to sterilize salmonids (including transgenic approaches to ablate the germ line of males or females), the most practical way to biologically contain salmonids is through the use of all-female triploid populations. This strategy produces fish with very little or no gonadal tissue, and the few eggs that may be produced are aneuploid and incapable of forming viable embryos. The advantage of using all-female triploids rather than mixed-sex populations is that males, although sterile, will develop gonadal tissue and undergo sexual maturation and would display reproductive behaviours upon return to spawning grounds to compete with fertile wild individuals. One problem that currently exists for the implemention of triploidy as a containment measure is that while 100% sterile populations can be produced consistently in laboratory trials, the inherent variation of the pressure or temperature shocking procedures and egg characteristics makes this difficult to achieve in large-scale production situations. Thus, it would be desirable to have further refinements to triploidy induction procedures to avoid the necessity of verifying the ploidy of each cultured transgenic fish to ensure sterility. As more data become available regarding the fitness of transgenic fish, the level of fertility present in triploid production populations may also be incorporated into future risk assessment exercises to ensure safe implementation of the technology.

## INTEGRATION OF BIOLOGICAL TECHNOLOGIES

While each aquaculture biotechnology is initially developed as a separate methodology, some of the most significant benefits can only be achieved by integration of two or more technologies. For example, a promising technique for sterility induction for aquaculture involves the production of fish that are both female and triploid (Donaldson et al. 1993*b*), and it was therefore necessary to integrate the production of monosex salmon with chromosome set manipulation techniques. Also, in order to use transgenic technologies in aquaculture it will be necessary to combine transgenesis with sterility induction. A further combination of technologies could be the linking of sterility, to maintain product quality throughout the year, with growth acceleration by somatotropin administration to maximize growth and feed conversion efficiency. This has been demonstrated on an experimental basis in salmonid culture (McLean et al. 1993*b*). In species where the ovary or testis has no significant inherent value, the sterile fast-growing organism may be ideal from both a quality and production standpoint. As new biotechnologies are developed and tested on a pilot scale, they will be incorporated into normal husbandry procedures where they will be evaluated and, ultimately, further refined for optimization of production.

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